Synthesis and Solid State Conformation of Tetrapeptide Amides Containing two Aib and two (Me)Phe Residues – Use of Enantiomerically Pure 2-Benzyl-2-methyl-2H-azirin-3-amines as (Me)Phe-Synthons

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Synthesis and Solid State Conformation of Tetrapeptide Amides Containing two Aib and two (αMe)Phe Residues – Use of Enantiomerically Pure 2-Benzyl-2-methyl-2*H*-azirin-3-amines as (αMe)Phe-Synthons

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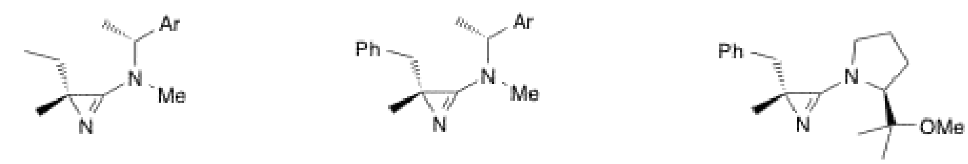
Dedicated to Professor Grzegorz Mlostoń on the occasion of his 70th birthday

A series of tetrapeptide amides containing two aminoisobutyric acids (Aib) and two α-methylphenylalanine ((αMe)Phe) units were prepared via the 'azirine/oxazolone method'. New 2-benzyl-2-methyl-2*H*-azirine-3-amines have been used for the selective introduction of (*S*)- and (*R*)-(αMe)Phe, respectively. The solid-state conformations of five tetrapeptide amides were determined by X-ray crystallography. In all cases, two β-turns stabilize 3_{10}-helical conformations and it was confirmed that, in contrast to proteinogenic amino acids, the configuration of (αMe)Phe does not determine the screw sense of the helix.

Keywords: 2*H*-azirin-3-amines • X-ray crystallography • α-methylphenylalanine • azirine/oxazolone method • peptide conformation

Introduction

Peptides containing 2-aminoisobutyric acid (Aib) units attract strong interest because of their chemical and biological properties. A well-known example is alamethicin,[1–5] a membrane-channel forming peptaibol[6–13] with antibiotic activities. We have shown that the 'azirine/oxazolone method' [14–18] is a convenient approach for the synthesis of Aib-containing oligopeptides[15, 19–24] and peptaibols.[25–32] In addition to Aib, peptaibols may also contain the chiral α,α-disubstituted α-amino acid isovaline (Iva).[33–38] In a series of studies of model peptides consisting of Aib and Iva residues[39,40] and of Iva homopeptides[41–43] it was shown that there is no unambiguous correlation between the configuration of Iva and the screw sense of the helical structure. Furthermore, α-methylphenylalanine ((αMe)Phe) has been studied intensely as a prototype of chiral α,α-disubstituted α-amino acids,[44–52] for example the influence of its configuration on the conformation of peptides.[53–59] For this reason, the synthesis of 3-amino-2*H*-azirines with two different substituents at C(2) and their use in peptide synthesis was an attractive challenge. Thus, the preparation of racemic synthons for Iva[60] and (αMe)Phe[61] was elaborated in our group and their convenient use in peptide synthesis has been demonstrated.[20,21,30,32,62–65] Furthermore, analogous optically active 3-amino-2*H*-azirines of type **1** – **3** (Fig. 1) bearing a chiral substituent at the amino group were obtained after chromatographic separation of the corresponding diastereomeric mixtures.[66–68]



(*R*,*R*)-**1a** (Ar = Ph)[66]
(*R*,*R*)-**1b** (Ar = 1-Naphthyl)[67]

(*R*,*R*)-**2a** (Ar = Ph)
(*R*,*R*)-**2b** (Ar = 1-Naphthyl)[67]

(*R*,*S*)-**3**[68]

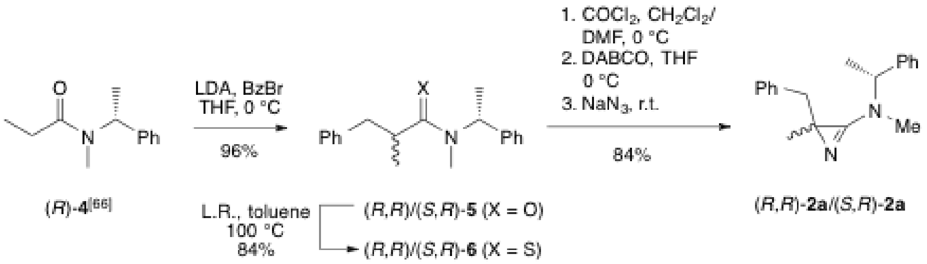
Figure 1. 3-Amino-2*H*-azirines as synthons for enantiomerically pure Iva and (α-Me)Phe.

The goal of the present study was the preparation of the enantiomerically pure (α-Me)Phe synthons (*R,R*)- and (*S,R*)-**2a** and their use in the synthesis of diverse tetrapeptide derivatives containing two Aib and two (αMe)Phe residues. Furthermore, the solid state conformations of the latter should be determined by X-ray crystallography.

Results and Discussion

Synthesis of Tetrapeptide Amides Containing two Aib and two (αMe)Phe Units.

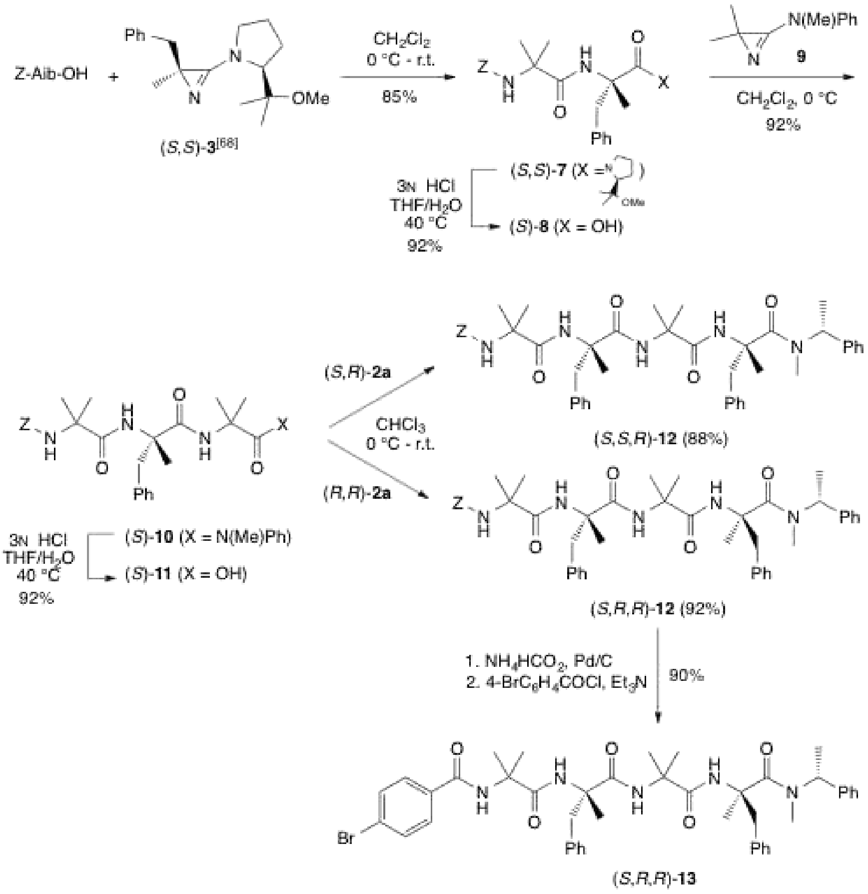
In analogy to the earlier published preparation of synthons for (*R*)- and (*S*)-(αMe)Phe of type **2**[67] or **3**[68], the desired 3-amino-2*H*-azirines (*R*,*R*)- and (*S*,*R*)-**2a** were synthesized as a ca. (1:1)-mixture by starting with the previously described propanamide (*R*)-**4**[66] (Scheme 1). Benzylation of (*R*)-**4** and subsequent reaction with Lawesson reagent (L.R.) led to a (1:1)-mixture of the thioamides (*R*,*R*)- and (*S*,*R*)-**6**, which could be separated chromatographically in small amounts for analytical purposes. Consecutive treatment of the mixture in CH_2Cl_2/DMF with $COCl_2$ (toluene solution), deprotonation with 1,4-diazabicyclo[2.2.2]octane (DABCO) in THF, and reaction with NaN_3 gave a (1:1)-mixture of the azirines (*R*,*R*)- and (*S*,*R*)-**2a**, which were separated chromatographically (MPLC). Based on the X-ray crystal structures of the subsequently prepared peptides, the configuration of the less polar diastereomer was elucidated as *S*,*R*.



Scheme 1. Synthesis of the 3-amino-2*H*-azirines (*R*,*R*)- and (*S*,*R*)-**2a** as (αMe)Phe synthons.

With the (αMe)Phe synthons **2a** and **3** in hand, a series of tetrapeptide derivatives with different sequences of two Aib and two (αMe)Phe units were prepared by using the 'azirine/oxazolone method'. The selected approach to Aib-(αMe)Phe-Aib-(αMe)Phe peptides with an alternating sequence of the two amino acids is shown in Scheme 2. Reaction of Z-Aib-OH with aminoazirine (*S*,*S*)-**3**,[68] followed by selective hydrolysis of the C-terminal amide group, gave the dipeptide (*S*)-**8** in high yield. Subsequent azirine coupling with the Aib-synthon **9** led to the tripeptide amide (*S*)-**10**, which was hydrolyzed to yield tripeptide (*S*)-**11**. The latter was coupled with the (αMe)Phe-synthons (*S*,*R*)-**2a** and (*S*,*S*)-**2a**, respectively, to give the desired tetrapeptide amides (*S*,*S*,*R*)-**12** and (*S*,*R*,*R*)-**12** in 88 and 92% yield, respectively.[1])

[1]) In preliminary experiments, the azirine coupling of (*S*)-**11** was also tested with the synthons (*S*,*S*)- and (*R*,*S*)-**3** in $CHCl_3$. The reactions occurred significantly slower and a considerable amount of decomposition products was formed.



Scheme 2. Synthesis of tetrapeptides **12** and **13** with alternating Aib and (αMe)Phe units by the 'azirine/oxazolone method'.

Whereas crystallization of (*S*,*S*,*R*)-**12** from AcOEt/hexane yielded crystals of good quality for X-ray crystallography, those of the diastereomer (*S*,*R*,*R*)-**12** were only weakly diffracting. For this reason and with the aim of testing a possible influence of the N-terminal carbonyl function on the conformation of the tetrapeptide, the N-terminus of (*S*,*R*,*R*)-**12** was deprotected by treatment with ammonium formate and Pd/C in boiling methanol.[69] The crude product was dissolved in AcOEt/Et_3N and reacted with 4-bromobenzoyl chloride to give (*S*,*R*,*R*)-**13** in 90% yield. Suitable crystals for the X-ray analysis of the latter were obtained from AcOEt/petroleum ether.

The synthesis of the next two tetrapeptide amides with two (αMe)Phe units inserted in positions two and three is presented in Scheme 3. Starting with dipeptide (*S*)-**8**, azirine coupling with the racemic (αMe)Phe-synthon *rac*-**14**[70] in CH_2Cl_2 occurred smoothly at room temperature to give a ca. (1:1)-mixture of the diastereomers (*S*,*R*)- and (*S*,*S*)-**15** in 96% yield.[2]) These two tripeptide amides were separated chromatographically (MPLC, AcOEt) and were obtained as pure diastereomers (*S*,*R*)-**15** and (*S*,*S*)-**15** in 35 and 37% yield, respectively. Based on the crystal structures of the subsequently prepared tetrapeptides, the diastereomer with the higher R_f-value is the (*S*,*R*) derivative.

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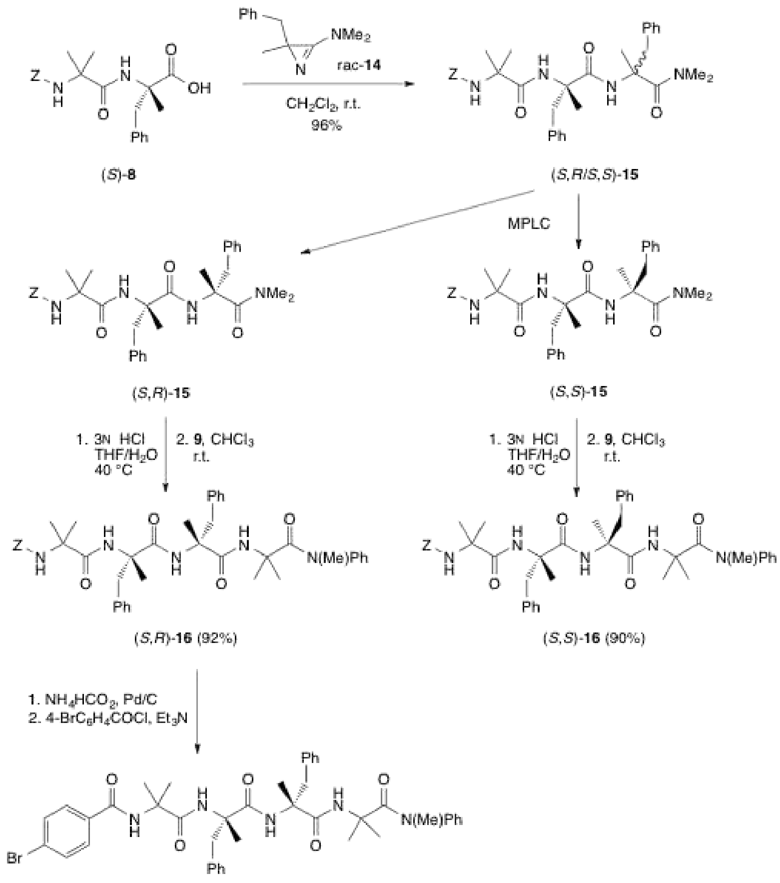
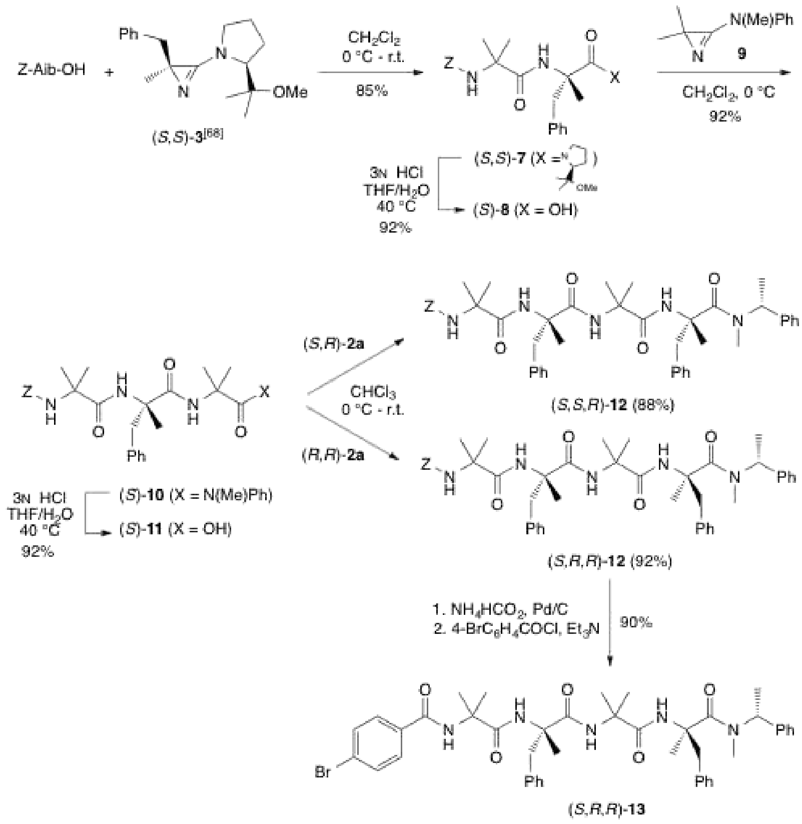
[2]) The attempted coupling of (*S*)-**8** with the (αMe)Phe-synthons (*S*,*S*)- and (*R*,*S*)-**3**, respectively, was unsuccessful. The azirines decomposed partially under the reaction conditions (CH_2Cl_2, 0 °C to r.t.). On the other hand, the tested coupling with (*S*,*R*)-**2a** and (*R*,*R*)-**2a**, respectively, in $CHCl_3$ at 40 °C was extremely slow and only small amounts of tripeptides were formed after several days.



Scheme 3. Synthesis of tetrapeptides **16** and **17** with accumulated (aMe)Phe units by the 'azirine/oxazolone method'.

Each of the two tripeptides **15** was hydrolyzed and coupled with the Aib-synthon **9** under the usual conditions to give the desired tetrapeptide amides (*S*,*R*)- and (*S*,*S*)-**16** in 92 and 90% yield, respectively. Crystallization of (*S*,*S*)-**16** from AcOEt/hexane led to suitable crystals for an X-ray structure determination. The diastereomer (*S*,*R*)-**16** was transformed into the 4-bromobenzoyl-protected derivative (*S*,*R*)-**17**, which also gave crystals of good quality.

Finally, two tetrapeptides with two Aib moieties in positions two and three and an (αMe)Phe unit in positions one and four were prepared according to Scheme 4. Reaction of 4-bromobenzoic acid with (*R*,*S*)-**3**[68] in THF led to the protected (αMe)Phe amide (*R*,*S*)-**18** in 45% yield. The molecular structure and configuration of this compound were established by X-ray single-crystal analysis (Fig. 2). The compound in the crystal is enantiomerically pure and the absolute configuration has been determined independently by the diffraction experiment. The molecules are linked by intermolecular N(1)–H···O(2') hydrogen bonds to form extended chains, which run parallel to the [001] direction and have a graph set motif[72] of C(5).



Scheme 4. Synthesis of tetrapeptides (*R*,*S*,*R*)- and (*R*,*R*,*R*)-**20** by the 'azirine/oxazolone method'.

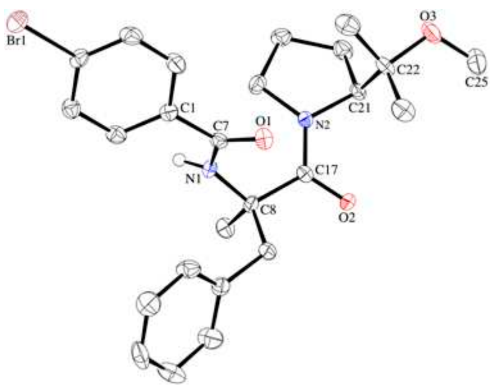


Figure 2. ORTEP Plot[71] of the molecular structure of (αMe)Phe derivative (*R*,*S*)-**18** (50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms have been omitted for clarity)

Selective hydrolysis of (*R*,*S*)-**18** under the usual conditions, subsequent coupling of the crude (αMe)Phe acid with azirine **9** as Aib synthon, again hydrolysis and coupling with **9** gave the tripeptide amide (*R*)-**19** in 77% yield. The latter was once more hydrolyzed and reacted with the (αMe)Phe synthons (*S*,*R*)- and (*R*,*R*)-**2a**, respectively, which lead to the desired tetrapeptide derivatives (*R*,*S*,*R*)- and (*R*,*R*,*R*)-**20** in 95 and 87% yield, respectively.

Crystal Structures of Tetrapeptide Amides Containing two Aib and two (αMe)Phe Units.

The structures and solid state conformations of three Aib-(αMe)Phe-Aib-(αMe)Phe derivatives and two Aib-(αMe)Phe-(αMe)Phe-Aib derivatives have been determined by single crystal X-ray analysis with the aim of comparing their conformations with those of known analogous peptides. For example, Toniolo and coworkers described the crystal structures of the tripeptides Z-(*S*)-(αMe)Phe-Aib-Aib-OH and Z-Aib-(*R*)-(αMe)Phe-Aib-O*t*Bu, the tetrapeptide Z-Aib-Aib-(*R*)-(αMe)Phe-Aib-O*t*Bu, and the pentapeptide 4-BrC_6H_4-CO-Aib-Aib-(*R*,*S*)-(αMe)Phe-Aib-Aib-O*t*Bu and concluded that (αMe)Phe strongly induces helical conformations.[55–59, 73] But in contrast to proteinogenic amino acids, the configuration of which determines the sense of the helical screw of the peptide (L(*S*)-configuration → right-handed helix (*P*)), this is not the case for (αMe)Phe, and Crisma and Toniolo summarized 'the reverse relationship between (αMe)Phe chirality and helix screw sense (L → left handed (*M*) and D → right handed (*P*)) prevails in the ratio 67% : 33%'.[73] Whereas the tetrapeptide 4-BrC_6H_4-CO-[(*R*)-(αMe)Phe]$_4$-O*t*Bu and the tripeptide 4-BrC_6H_4-CO-[(*R*)(αMe)Phe]$_3$-OH crystallize as right-handed helices,[73] the dipeptide amide 4-BrC_6H_4-CO-[(*R*)-(αMe)Phe]$_2$-NHMe forms a left-handed β-turn of type III'.[57] In the case of the pentapeptide 4-BrC_6H_4-CO-(*R*)-(αMe)Phe-Aib-Aib-(*R*)-(αMe)Phe-Aib-O*t*Bu containing two (*R*)-(αMe)Phe units, the molecules in the crystalline state form right-handed 3_{10}-helical structures stabilized by three intramolecular hydrogen bonds.[55]

The crystals of the tetrapeptide (*S*,*S*,*R*)-**12** are enantiomerically pure, but the absolute configuration of the molecule has not been determined by crystallographic methods. The enantiomer used in the refinement was based on the known (5*S*,14*R*)-configuration of the molecule. Based on this assumption, the configuration at C(11) is *S*. The molecule forms a 3_{10}-helix, which is held in place by two intramolecular hydrogen bonds (Fig. 3, Table 1). N(7)–H and N(10)–H interact with the carbamate O(21)- and the amide O(3)-atom, respectively, forming formal ten-membered rings (β-turns, graph set motif[72] S(10)). The other two amide NH groups are involved in intermolecular hydrogen bonds: N(1)–H forms a H-bond with the amide O(12') at the opposite end of a neighboring molecule and N(4)–H forms a weak H-bond with the same O(12'). These two intermolecular interactions link the molecules into extended chains, which run parallel to the [001] direction and can be described by the graph set motifs C(14) and C(11), respectively. The conformation of the tetrapeptide derivative is characterized by two consecutive β-turns of type III, i.e. it corresponds with a right-handed 3_{10}-helical structure with torsion angles ϕ_{1-3} and ψ_{1-3} (Table 2) in accordance with those of an ideal 3_{10}-helix ($\phi = -60°$, $\psi = -30°$). This result is remarkable in comparison with the pentapeptide 4-BrC_6H_4-CO-(*R*)-(αMe)Phe-Aib-Aib-(*R*)-(αMe)Phe-Aib-O*t*Bu described by Toniolo et al.[55] Although the configurations of the two incorporated (αMe)Phe units are opposite, right-handed helices are formed in both cases. It is also worth mentioning that in the previously described pentapeptide Z-Gly-Aib-(*RS*)-(αMe)Phe-Aib-Gly-OMe the molecule containing (*S*)-(αMe)Phe forms a left-handed 3_{10}-helix.[65]

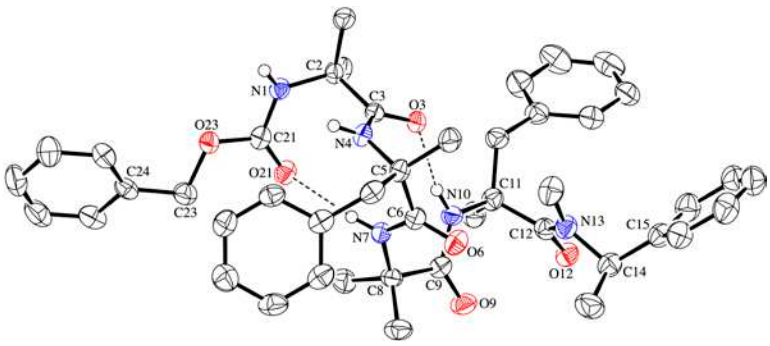


Figure 3. ORTEP Plot[71] of the molecular structure of the tetrapeptide derivative (*S*,*S*,*R*)-**12** (50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms have been omitted for clarity)

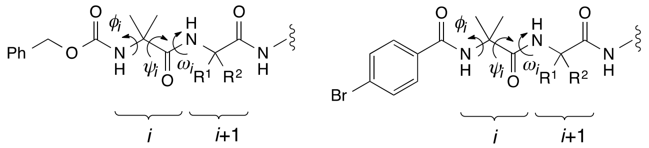
Table 1. Intramolecular hydrogen bonds of tetrapeptide derivatives (*S*,*S*,*R*)-**12**, (*S*,*R*,*R*)-**12**, (*S*,*R*,*R*)-**13**, (*S*,*S*)-**16**, and (*S*,*R*)-**17** (atom numbering refers to Figs. 3 – 7)

Compound	N(7)–H···O(21)/O(22) N···O (Å)	N–H···O (°)	N(57)–H···O(70)[a] N···O (Å)	N–H···O (°)	N(10)–H···O(3) N···O (Å)	N–H···O (°)	N(60)–H···O(53)[a] N···O (Å)	N–H···O (°)
(*S*,*S*,*R*)-**12**	3.063(4)	164(3)			2.911(4)	168(3)		
(*S*,*R*,*R*)-**12**	3.145(8)	155	3.198(8)	148	3.055(7)	169	3.033(7)	162

(*S*,*R*,*R*)-**13**	3.159(10) 166		2.987(8) 166	
(*S*,*S*)-**16**	2.917(4) 165(4)	3.045(4) 162(5)	2.945(4) 164(3)	2.887(4) 161(4)
(*S*,*R*)-**17**	3.030(7) 148	2.961(6) 151	3.018(6) 141	2.935(6) 151

[a] In those structures with two symmetry-independent molecules

Table 2. Torsion Angles ϕ, ψ, and ω of the backbone of tetrapeptide derivatives (*S*,*S*,*R*)-**12**, (*S*,*R*,*R*)-**12**, (*S*,*R*,*R*)-**13**, (*S*,*S*)-**16** and (*S*,*R*)-**17**



	(*S*,*S*,*R*)-**12**	(*S*,*R*,*R*)-**12**	(*S*,*R*,*R*)-**13**	(*S*,*S*)-**16**	(*S*,*R*)-**17**
ϕ_1	–55.9(4)	–51.5(8); –55.3(8)	–58(1)	57.3(4); 58.0(5)	55.3(7); –54.7(7)
ψ_1	–33.3(4)	–39.6(7); –39.5(8)	–32(1)	28.5(4); 25.1(4)	36.8(7); –33.7(7)
ω_1	–175.7(3)	–170.9(5); –170.3(6)	–173.3(7)	–177.7(3); –179.8(3)	177.1(5); –176.4(5)
ϕ_2	–57.6 (4)	–54.0(7); –60.5(8)	–57(1)	52.8(4); 51.2(4)	49.1(7); –53.0(7)
ψ_2	–24.4(4)	–34.1(7); –31.1(8)	–23(1)	33.3(4); 34.3(4)	41.4(7); –34.9(7)
ω_2	179.5(3)	–171.9(6); –174.7(6)	–179.5(7)	179.6(3); 178.5(3)	172.5(5); –176.7(5)
ϕ_3	–64.8(4)	–84.5(8); –79.0(8)	–67(1)	63.7(4); 64.4(4)	64.8(7); –61.2(7)
ψ_3	–26.8(4)	–7.2(9); –8.4(9)	–16(1)	17.7(5); 19.8(4)	25.0(7); –25.9(7)
ω_3	–170.8(3)	–177.1(6); –177.3(6)	179.5(7)	–172.8(3); –180.0(3)	–171.2(5); 175.5(5)
ϕ_4	–52.9(4)	47.9(8); 50.4(8)	58(1)	–50.1(4); –55.2(4)	–46.5(8); 49.5(8)
ψ_4	–51.9(4)	47.2(8); 47.9(9)	49.7(9)	–51.4(5); –50.8(4)	–57.2(8); 56.8(8)
ω_4	167.2(3)	176.7(6); 179.2(6)	–178.9(7)	178.5(3); –179.9(3)	–173.5(5); 165.4(5)

Also the crystals of (*S*,*R*,*R*)-**12** are enantiomerically pure; the enantiomer used in the refinement was based on the known *S*-configuration of C(5). Therefore, the configurations of C(11) and C(14) are both *R*. The asymmetric unit contains two symmetry-independent molecules of the peptide plus two AcOEt molecules. Peptide molecule B exhibits disorder of the terminal methylbenzyl group and of the terminal O-benzyl ring. The solvent molecules are also disordered. The torsion angles (Table 2) show that the backbones of the two independent peptide molecules have virtually identical conformations and the same absolute configuration. The main differences between the molecules are small twists in the orientations of some of the phenyl groups. Both molecules form helical structures (Figure 4, molecule A) similar to (*S*,*S*,*R*)-**12**,but the first β-turn of type III is followed by a type I β-turn (ϕ_3 ≈ –90°, ψ_3 ≈ 0°). The torsion angles ϕ_4 and ψ_4 of the last amino acid, (*R*)-(αMe)Phe, are inverted compared with those of (*S*,*S*,*R*)-**12**. In each molecule, two intramolecular H-bonds (N(7)–H···O(21) and N(10)–H···O(3), graph set motif S(10), Table 1) are formed in analogy to (*S*,*S*,*R*)-**12**. Intermolecular H-bonds between N(4)–H and the amide O(12') atom at the opposite end of a neighboring molecule A link the molecules of type A into extended chains, which run parallel to the [100] direction (graph set motif C(11)). Remarkably, the carbamate N(1)–H does not form any hydrogen bond. Molecules B exhibit the same pattern of interactions as molecules A, except that the carbamate N(51)–H forms an intermolecular H-bond with O(62') of a neighboring type B molecule, while N(54)–H is not involved in any hydrogen bonds. Once again, the intermolecular interactions form extended chains, which run parallel to the [100] direction, but in this case the graph set motif is C(14).

a) b)

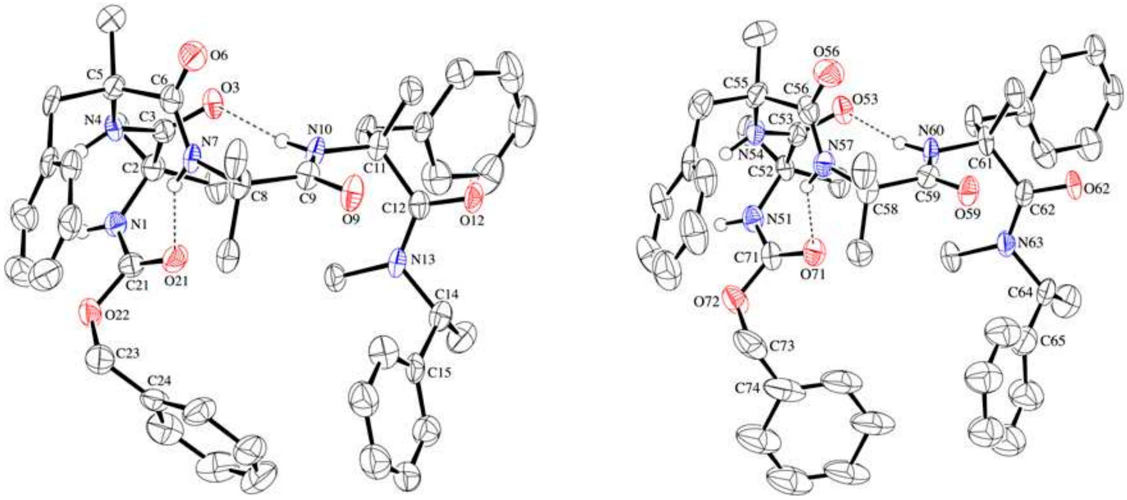


Figure 4. ORTEP Plots[71] of the molecular structures of the tetrapeptide derivative (*S*,*R*,*R*)-**12** (a) molecule A and b) one conformation of molecule B; 50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms have been omitted for clarity)

In contrast to the Z-protected tetrapeptide (*S*,*R*,*R*)-**12**, the derivative (*S*,*R*,*R*)-**13** with a terminal 4-bromobenzoyl group exists as a uniform conformer. The compound in the crystal is enantiomerically pure and its absolute configuration (5*S*,11*R*,14*R*) has been determined independently by the diffraction experiment. The asymmetric unit contains one peptide molecule (Figure 5) plus one disordered AcOEt molecule. The peptide molecules form helices, which are held in place by two intramolecular H-bonds analogous to (*S*,*S*,*R*)-**12** and (*S*,*R*,*R*)-**12** (N(7)–H….O(21) and N(10)–H….O(3), Table 1; graph set motif S(10)). The torsion angles ϕ_{1-3} and ψ_{1-3} are again very similar to those of (*S*,*S*,*R*)-**12** and ϕ_4 and ψ_4 are inverted, analogous to the case of (*S*,*R*,*R*)-**12** (Table 2). In contrast to (*S*,*R*,*R*)-**12**, the values of ϕ_3 and ψ_3 are close to the ideal values of a β-turn type III. Therefore, the peptide backbone of (*S*,*R*,*R*)-**13** forms two consecutive β-turns of type III, i.e. a 3_{10}-helical conformation. The comparison of (*S*,*R*,*R*)-**12** and (*S*,*R*,*R*)-**13** shows that the N-terminal protecting group (Z or 4-Br-C_6H_4CO) has some influence on the peptide-conformation. Similar to molecule B of (*S*,*R*,*R*)-**12**, N(1)–H forms an intermolecular H-bond with the amide O(12’) atom of a neighboring molecule leading to extended chains, which run parallel to the [010] direction (graph set motif C(14)). N(4)–H is not involved in any hydrogen bonds.

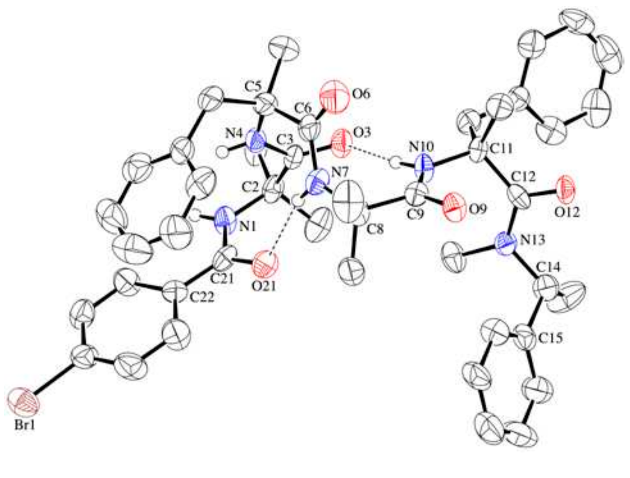


Figure 5. ORTEP Plot[71] of the molecular structure of the tetrapeptide derivative (*S*,*R*,*R*)-**13** (50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms have been omitted for clarity)

The space group of the crystals of tetrapeptide (*S*,*S*)-**16** also permits the compound to be enantiomerically pure, but the absolute configuration of the molecule has not been determined. The enantiomer used in the refinement was based on the known *S*-configuration of C(5). There are two symmetry-independent peptide molecules in the asymmetric unit plus one water molecule with full site occupancy and a second water molecule, which seems to be present only at ca. 30% of its sites. Both peptide molecules A and B (Figure 6) form left-handed helices, which are held in place by two intramolecular H-bonds, as in the previous cases (N(7)–H···O(20), N(10)–H···O(3) and N(57)–H….O(70), N(60)–H….O(53), respectively; Table 1; graph set motif S(10)). The two independent peptide molecules have virtually identical backbone conformations and the same absolute configuration. The torsion angles (Table 2) correspond with two consecutive β-turns of type III'. Again it is worth mentioning that left-handed helical structures are formed although two *S*-configured (αMe)Phe units are included in positions 2 and 3, whereas in (*S*,*S*,*R*)-**12**, with two (*S*)-(αMe)Phe units in positions 2 and 4, a right-handed helix is exhibited.

Again, N(1)–H forms an intermolecular H-bond with the amide O(12') atom of a neighboring molecule A. These interactions link the molecules of type A into extended chains, which run parallel to the [001] direction and can be described by a graph set motif of C(14). Molecules B exhibit exactly the same pattern of interactions as molecule A, except that the chains run parallel to the [100] direction. N(4)–H donates a H-bond to the partial occupancy water molecule while O(12) probably accepts a H-bond from a different water molecule. Thus this water molecule is part of the linking process in the chains of molecule A. Similarly, N(54)–H of molecule B donates to the full occupancy water molecule, but this water molecule appears to be a H-bond donor to O(71) as well as to O(59) of an adjacent molecule. In this way, this water molecule is also involved in the linking of the type B molecules into chains.

a) b)

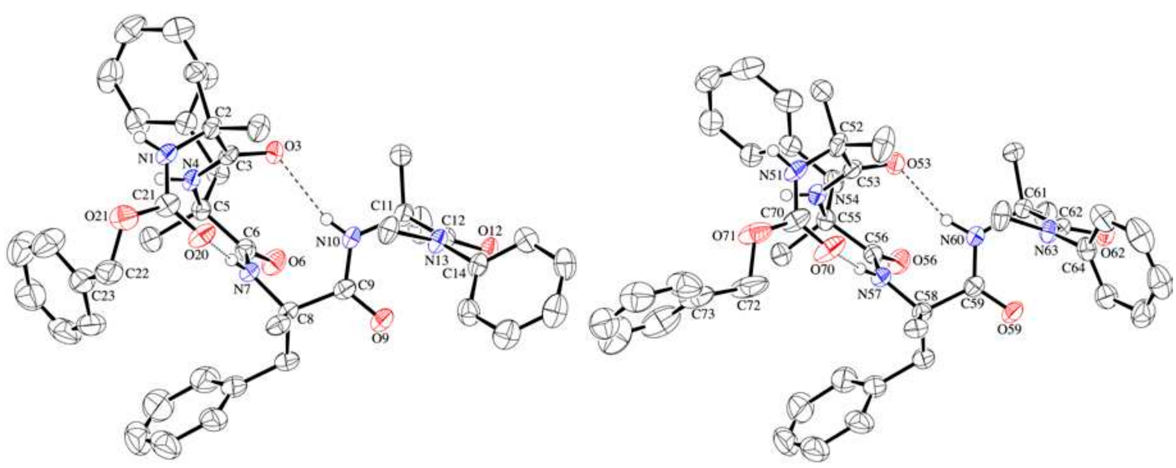


Figure 6. ORTEP Plots[71] of the molecular structures of the two symmetry-independent molecules of tetrapeptide derivative (*S*,*S*)-**16** (50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms have been omitted for clarity)

Finally, crystal structure analysis of the tetrapeptide derivative (*S*,*R*)-**17** shows that the compound is enantiomerically pure and the absolute configuration has been determined independently by the diffraction experiment. The molecule has the (5*S*,8*R*)-configuration. The asymmetric unit contains two independent peptide molecules plus one ordered and one disordered AcOEt molecule. As in the other cases, the peptide molecules form helices of type 3_{10} (Figure 7), i.e. two consecutive β-turns of type III/III' with two intramolecular hydrogen bonds in each case (N(7)–H···O(20), N(10)–H···O(3) and N(57)–H···O(70), N(60)–H···O(53), respectively; Table 1, graph set motif S(10)). The torsion angles (Table 2) show that the backbones of the two independent molecules with the same absolute configuration have almost perfect mirror image conformations. Whereas in molecule A two β-turns of type III' form a left-handed helical structure, two β-turns of type III stabilize a right-handed helical structure in molecule B. In addition, the phenyl groups at C(8) and C(58), respectively, have different orientations.

a) b)

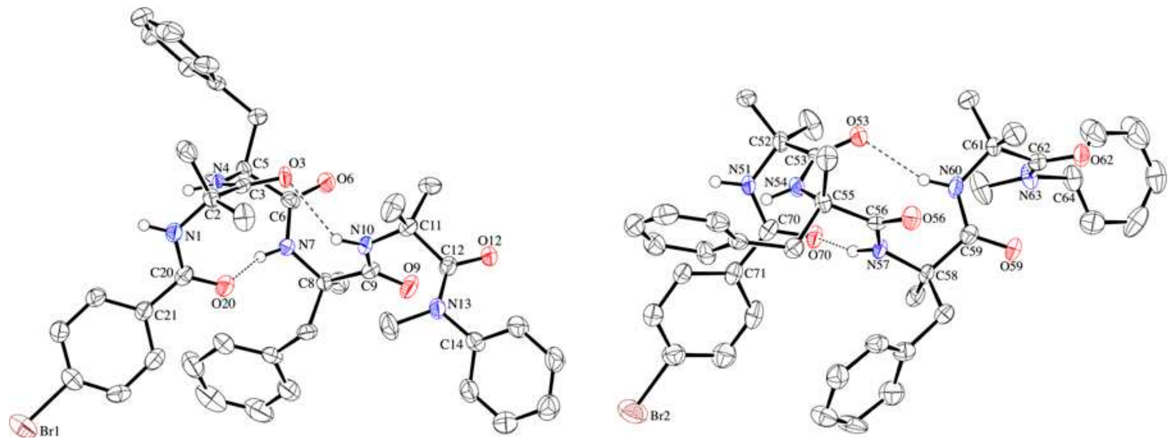


Figure 7. ORTEP Plots[71] of the molecular structures of the two symmetry-independent molecules of tetrapeptide derivative (*S*,*R*)-**17** (50% probability ellipsoids; arbitrary numbering of atoms; H-atoms bonded to C-atoms have been omitted for clarity)

The pattern of the intermolecular hydrogen bonds is again similar to that in the peptides describe above. N(1)–H of molecule A forms a H-bond with the amide O(12') atom of a neighboring molecule A, thereby linking the molecules into extended chains, which run parallel to the [001] direction. N(4)–H interacts with the O-atom of the disordered AcOEt molecule. The same H-bond pattern is observed for molecule B (N(51)–H···O(62')), except that N(54)–H is not involved in any hydrogen bonds.

Conclusions

With the presented synthesis of the 3-amino-2*H*-azirines (*R*,*R*)- and (*S*,*R*)-**2a**, useful new synthons for enantiomerically pure (αMe)Phe are available. It has been shown that these building blocks are more reactive than the earlier described derivatives (*S*,*S*)- and (*R*,*S*)-**3**,[68] which is of importance for their application in peptide synthesis via the 'azirine/oxazolone method'. Even more reactive is the racemic (αMe)Phe synthon rac-**14** bearing a dimethylamino group at C(3). Obviously, the type and bulkiness of the N-substituents play a significant role. By using these three (αMe)Phe synthons in the 'azirine/oxazolone method', three types of N-protected tetrapeptide amides containing two (αMe)Phe and two Aib units were prepared successfully.

The solid-state conformations of five of these tetrapeptides have been determined by X-ray diffraction analysis. As expected for peptides containing α,α-disubstituted α-amino acids, all peptides adopt helical conformations held in place by two intramolecular hydrogen bonds to form two consecutive β-turns. Furthermore, the statement of Crisma and Toniolo[73] that, in contrast to proteinogenic amino acids, the configuration of (αMe)Phe does not determine the screw sense of the helix unambiguously has been confirmed. Thus, the tetrapeptide (*S*,*S*,*R*)-**12** containing two *S*-configured (αMe)Phe units forms a right-handed 3_{10}-helix very similar to that of 4-BrC_6H_4-CO-(*R*)-(αMe)Phe-Aib-Aib-(*R*)-(αMe)Phe-Aib-O*t*Bu with two *R*-configured (αMe)Phe units.[55] Also (*S*,*R*,*R*)-**12** and (*S*,*R*,*R*)-**13**, which contain one *S*- and one *R*-configured (αMe)Phe, adopt a right-handed helical structure, but the C-terminal (αMe)Phe unit is twisted. Furthermore, the kind of the N-terminal protecting group, Z or 4-BrC_6H_4-CO, has little influence on the backbone conformation. Surprisingly, peptide (*S*,*S*)-**16**, which possesses two (*S*)-(αMe)Phe inserted in positions 2 and 3, exists in the crystal as left-handed 3_{10}-helices, in contrast to (*S*,*S*,*R*)-**12** with the two (*S*)-(αMe)Phe residues in positions 2 and 4. Obviously, the position of (αMe)Phe in the peptide sequence also plays a crucial role in the determination of the sense of the helical screw. Even more astonishing is the presence of a 1:1 mixture of left- and right-handed 3_{10}-helices in the case of (*S*,*R*)-**17**, which has (*S*)-(αMe)Phe in position 2 and (*R*)-(αMe)Phe in position 3 of the tetrapeptide.

Experimental Section

General

See refs. [66, 68, 74] The used 3-amino-2*H*-azirines (*S*,*S*)- and (*R*,*S*)-**3** ((*S*,*S*)- and (*R*,*S*)-1-(2-benzyl-2-methyl-2*H*-azirin-3-yl)-2-(1-methoxy-1-methylethyl)pyrrolidine)[68], **9** (2,2,*N*-trimethyl-*N*-phenyl-3-amino-2*H*-azirine),[61,62] and rac-**14** ((*R*/*S*)-2-benzyl-2,*N*,*N*-trimethyl-3-amino-2*H*-azirine),[70] as well as (*R*)-*N*-methyl-*N*-(1-phenylethyl)propanamide ((*R*)-**4**),[66] were prepared previously. All other chemicals were commercially available. If not otherwise indicated, IR spectra in KBr, ^{1}H- and ^{13}C-NMR spectra in $CDCl_3$ at 300 and 75 MHz, respectively.

*General Procedure 1 (GP 1, Coupling with 2*H*-azirin-3-amines).* To a solution of an N-protected amino acid or N-protected peptide in CH_2Cl_2 at 0°, a solution of the corresponding 2*H*-azirin-3-amine in CH_2Cl_2 was added and the mixture was stirred at r.t. for several h. After completion of the reaction, the solvent was evaporated and the product was purified chromatographically (SiO_2, AcOEt/hexane) or by crystallization. In some cases, the product was obtained as an amorphous solid.

General Procedure 2 (GP 2, Selective hydrolysis of peptide amides). To a solution of the peptide amide in THF at r.t., the same volume of 6N HCl was added and the mixture stirred at r.t. until the starting material disappeared. Then, CH_2Cl_2 was added and the mixture was extracted with 1N HCl and saturated aqueous NaCl solution. The organic layer was dried ($MgSO_4$) and the solvent evaporated. The product was precipitated or purified chromatographically (SiO_2).

*Synthesis of 2-benzyl-2-methyl-3-[*N*-methyl-*N*-(1-phenylethyl)amino]-2*H*-azirine* ((*R*,*R*)- and (*S*,*R*)-**2a**

*(*R*)-2-Benzyl-*N*-methyl-N-(1-phenylethyl)propanamide* ((*R*,*R*)/(*S*,*R*)-**5**). Analogous to ref.[74], to a solution of 12.9 g (67.4 mmol) of (*R*)-*N*-methyl-*N*-(1-phenylethyl)propanamid ((*R*)-**4**) in 100 mL of THF was added 58 mL of a 1.5N solution of LDA at 0 °C. Then, 11.8 g (69.0 mmol) of benzylbromide were added and the mixture was stirred at 0 °C for 2 h. After addition of aqueous 2N HCl, extraction with Et_2O, and column chromatography (CC, SiO_2, hexane/AcOEt 1:1), 18.2 g (96%) of a ca. 2:1 mixture of two diastereomers of **2a** were obtained. IR (neat): 3020m, 2965s, 2925m, 1635s, 1490m, 1450m, 1400m, 1080m, 740m, 730m, 700s. ^{1}H-NMR (2 diastereomers + rotamers): 7.35–7.15, 6.95–6.9, 6.75–6.7 (3m, 10 arom. H), 6.1–5.9, 5.15–5.05, 5.0–4.95 (3m, NC*H*MePh), 3.2–3.0, 2.70 (m and dd, *J* = 11.8, 4.5, PhC*H*$_2$, MeC*H*CO), 2.59, 2.56, 2.47, 2.39, 2.34 (5s, NMe), 1.55, 1.42, 1.29, 1.25, 1.21, 1.09 (6d, *J* = ca. 7, 2 Me). ^{13}C-NMR: 175.8, 175.7 (2s, CO), 140.8, 140.3, 140.2 (3s, 2 arom. C), 129.2,129.0, 128.6, 128.3, 128.2, 128.2, 127.1, 126.8, 126.3, 126.2, 126.1, 126.1, 125.2 (14d, 10 arom. CH), 54.4, 54.2, 50.0 (3d, N*C*HMePh), 41.2, 40.6, 40.4 (3t, Ph*C*H$_2$), 38.6, 38.4 (2q, NMe), 29.4, 29.1, 28.1 (3d, Me*C*HCO), 18.7, 17.8, 17.2 (3q, NCH*Me*Ph), 15.5, 15.2 (2q, *Me*CHCO). EI-MS: 281 (97, $M^{+\cdot}$). 266 (14), 162 (29), 120 (51, [HNCHMePh]$^+$), 119 (35), 118 (13), 105 (96, [CHMePh]$^+$), 103 (12), 91 (100, [C_7H_7]$^+$), 77 (25). Anal. calc. for $C_{19}H_{23}NO$ (281.40): C 81.10, H 8.24, N 4.98; found: C 81.41, H 8.46, N 4.97.

*(*R*)-2-Benzyl-*N*-methyl-N-(1-phenylethyl)propanethioamide* ((*R*,*R*)/(*S*,*R*)-**6**). A solution of 18.1 g (64.3 mmol) (*R*,*R*)/(*S*,*R*)-**5** and 14.3 g Lawesson reagent (LR) in 100 mL of toluene was heated to 100 °C for 18 h. Evaporation of the solvent and chromatographic workup (CC, SiO_2, hexane/AcOEt 10:1) gave 16.0 g (84%) of **6** as a mixture of diastereoisomers. IR ($CHCl_3$): 3060w, 2970s, 2925s, 1600w, 1490s, 1480s, 1450s, 1410s, 1380m, 1310m, 1260s, 1090s, 1060s, 990m, 970m, 700s, 660m. CI-MS: 298 ([M+H]$^+$). Anal. calc. for $C_{19}H_{23}NS$ (297.46): C 76.72, H 7.79, N 4.71, S 10.78; found: C 76.57, H 7.76, N 4.60, S 10.52.

A small amount of the mixture was separated by TLC (SiO_2, hexane/AcOEt 10:1). Diastereomer **6a** (R_f = 0.25): M.p. 101–103 °C. $[\alpha]_D$ +335.4 ($CHCl_3$, c = 0.999). ^{1}H-NMR (2 rotamers ca. 2:1): 7.35–7.15 (m, 10 arom. H), 6.7–6.65, 5.65–5.55 (2m, NC*H*MePh), 3.65–3.6, 3.45–3.2 (2m 1 H of PhC*H*$_2$ and MeC*H*CO), 2.95–2.8 (m, 1 H of PhC*H*$_2$), 3.02, 2.54 (2s, NMe), 1.61, 1.36, 1.33, 1.31 (4d, *J* = ca. 7, 2 Me). ^{13}C-NMR: 209.7, 209.2 (2s, CS), 140.2, 139.9, 139.2, 138.6 (4s, 2 arom. C), 129.4, 129.2, 128.7, 128.5, 128.4, 128.2, 127.7, 127.5, 126.8, 126.4, 126.3, 126.2 (12d, 10 arom. CH), 58.1 (d N*C*HMePh), 44.6 (q, NMe), 44.2 (t, Ph*C*H$_2$), 37.8, 33.3 (2d, Me*C*HCO), 22.6, 21.7, 17.5, 14.0 (4q, 2 Me).

Diastereomer **6b** (R_f = 0.3): $[\alpha]_D$ +107.8 ($CHCl_3$, c = 1.009). ^{1}H-NMR (2 rotamers ca. 3:1): 7.35–7.05, 6.85–6.8 (m, 10 arom. H and 0.75 H of NC*H*MePh), 5.5–5.45 (m, 0.25 H of NC*H*MePh), 3.65–3.6, 3.45–3.5, 3.35–3.25 (3m, 1 H of PhC*H*$_2$ and MeC*H*CO), 2.95–2.8 (m, 1 H of PhC*H*$_2$), 3.04, 2.66 (2s, NMe), 1.48, 1.43, 1.34, 1.10 (4d, *J* = ca. 7, 2 Me). ^{13}C-NMR: 209.5 (s, CS), 140.2, 139.9, 139.1, 138.8 (4s, 2 arom. C), 129.4, 129.2, 128.9,128.5, 128.3, 127.8, 127.3, 126.8, 126.4, 126.3, 126.2 (11d, 10 arom. CH), 58.4 (d, N*C*HMePh), 44.8, 43.9 (2t, Ph*C*H$_2$), 44.6, 44.6 (2q, NMe), 37.7, 33.8 (2d, Me*C*HCO), 23.0, 21.8, 17.0, 14.1 (4q, 2 Me).

*(*R,R*)- and (*S,R*)-2-Benzyl-2-methyl-3-(*N*-methyl-*N*-(1-phenylethyl)amino-2*H*-azirine* ((*R*,*R*)- and (*S*,*R*)-**2a**). To a solution of 6.45 g (21.7 mmol) (*R*,*R*)/(*S*,*R*)-**6** in 25 mL of CH_2Cl_2 and 3 drops of DMF were added 12 mL of a 2N phosgene ($COCl_2$) solution in toluene at 0 °C. The mixture was stirred at 0 °C for 1 h. Then, the solvent was evaporated, the residue dissolved in 30 mL of THF and 2.43 g of 1,4-

diazabicyclo[2.2.2]octane (DABCO) were added at 0 °C. After 30 min, the mixture was filtered and the filtrate was treated with 3 g (46.1 mmol) NaN_3 at r.t. over night. After filtration via Celite and extraction with CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution, the solvent was evaporated and the residue purified by chromatography (CC followed by MPLC, SiO_2, hexane/AcOEt 1:1) to give 4.85 g (80%) of (*R*,*R*)/(*S,R*)-**2a** as a ca. 1:1 mixture of diastereomers. IR (neat): 3080w, 3060w, 3020w, 2965m, 2930m, 2900m, 1760s, 1600w, 1445m, 1370m, 1240m, 1075m, 760w, 700s. CI-MS: 279 (100, $[M+1]^+$), 277 (18, $[M–1]^+$), 173 (53).

The diastereomers were separated by TLC (SiO_2, hexane/AcOEt 1:1) and obtained in nearly equal amounts. (*S*,*R*)-**2a** (R_f = 0.35)[3]): $[\alpha]_D$ +1.18 ($CHCl_3$, c = 0.997). ^{1}H-NMR ((D_6)DMSO, 363 K): 7.4–7.15 (m, 10 arom. H), 4.64 (q, *J* = ca. 7, NC*H*MePh), 2.89, 2.81 (AB, *J* = 14.3, PhCH_2), 2.67 (s, NMe), 1.48 (d, *J* = ca. 7, NCH*Me*Ph), 1.22 (s, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.9 (s, C(3)), 141.4, 139.0 (2s, 2 arom. C), 129.8, 128.8, 128.1, 127.6, 127.0, 126.2 (6d, 10 arom. CH), 58.5 (d, N*C*HMePh), 44.7 (t, Ph*C*H_2), 42.8 (q, NMe), 33.0 (s, C(2)), 23.7 (q, NCH*Me*Ph), 18.2 (q, Me). Anal. calc. for $C_{19}H_{22}N_2$ (278.40): C 82.09, H 7.97, N 10.06; found: C 81.88, H 7.90, N 10.06.

(*R*,*R*)-**2a** (R_r = 0.3): $[\alpha]_D$ +78.75 ($CHCl_3$, c = 1.021). ^{1}H-NMR ((D_6)DMSO, 363 K): 7.35–7.15 (m, 10 arom. H), 4.60 (q, *J* = ca. 7, NC*H*MePh), 2.82 (s, PhCH_2), 2.67 (s, NMe), 1.48 (d, *J* = ca. 7, NCH*Me*Ph), 1.23 (s, Me). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.8 (s, C(3)), 141.4, 139.1 (2s, 2 arom. C), 129.8, 128.8, 128.2, 127.6, 126.9, 126.2 (6d, 10 arom. CH), 58.6 (d, N*C*HMePh), 44.8 (t, Ph*C*H_2), 43.1 (q, NMe), 32.4 (s, C(2)), 23.9 (q, NCH*Me*Ph), 18.0 (q, Me).

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[3]) X-ray crystal structure determinations of the peptides prepared using the diastereomer with higher R_f value showed that the less polar isomer has *S,R*-configuration.

Synthesis of tetrapeptide amides (*S*,*S*,*R*)-**12**, (*S*,*R*,*R*)-**12**, and (*S*,*R*,*R*)-**13** *with alternating Aib and (αMe)Phe units*

*(*S,S*)-*N*-(Benzyloxycarbonyl)-*N'*-{1-benzyl-1-methyl-2-[2-(1-methoxy-1-methylethyl)pyrrolidin-1-yl]-2-oxoethyl}aminoisobutanamide* ((*S*,*S*)-Z-Aib-(αMe)Phe-[2-(1-methoxy-1-methylethyl)pyrrolidid], (*S*,*S*)-**7**). To a solution of 119 mg (0.5 mmol) Z-Aib-OH in 3 mL of CH_2Cl_2 were added 158 mg (0.55 mmol) of azirine (*S*,*S*)-**3** dissolved in 2 mL of CH_2Cl_2 and the mixture was stirred at r.t. for 4 d. Evaporation of the solvent and chromatographic workup (CC, SiO_2, hexane/AcOEt 1:1) gave 220 mg (84%) of (*S*,*S*)-**7**. M.p. 151–152 °C. $[\alpha]_D$ –14.9 ($CHCl_3$, c = 0.525). IR: 3360s, 3300s, 2970s, 2940m, 1715s, 1660s, 1610s, 1500s, 1430s, 1395m, 1325s, 1220m, 1200m, 1080s, 1055s, 700s, 655m. ^{1}H-NMR: 7.54 (s, NH), 7.35–7.3, 7.25–7.15 (2m, 10 arom. H), 5.46 (br s, NH), 5.07, 5.03 (AB, *J* = 14.4, PhCH_2), 3.4–3.35 (m, 1 H), 3.17 (s, MeO), 2.0–1.9 (m, 2 H), 1.65 (br s, 2 H), 1.57, 1.47, 1.13, 0.98 (4s, 5 Me). ^{13}C-NMR: 172.3, 171.4 (2s, 2 amide CO), 154.8 (s, OCONH), 136.3, 130.1 (2s, 2 arom. C), 130.1, 128.4, 128.1, 128.0, 126.8 (5d, 10 arom. CH), 78.6 (s, Me_2*C*OMe), 66.4 (t, Ph*C*H_2O), 65.0 (d, NCH), 61.0, 57.0 (2s, N*C*MeCH_2Ph and N*C*Me_2), 49.0 (q, MeO), 48.3, 41.3 (2t, 2 CH_2), 25.6 (q, Me), 25.3 (t, CH_2), 25.0 (q, Me), 23.8 (t, CH_2), 23.0, 22.7, 22.1 (3q, 3 Me). CI-MS: 524 ($[M+1]^+$). Anal. calc. for $C_{30}H_{41}N_3O_5$ (523.68): C 68.81, H 7.89, N 8.02; found: C 68.87, H 7.83, N 8.03.

*Z-Aib-(*S*)-(αMe)Phe-OH* ((*S*)-**8**). According to *GP 2*, a solution of 1.00 g (1.91 mmol) (*S*,*S*)-**7** in 15 mL of THF was treated with 15 mL of 6N HCl at 40 °C overnight. Chromatography (CC, SiO_2, AcOEt) and precipitation of the product by addition of Et_2O/hexane yielded 0.723 g (ca. 92%) of (*S*)-**8**, contaminated with butane-1,4-diol. TLC (SiO_2, AcOEt) led to pure (*S*)-**8**. $[\alpha]_D$ +24.95 ($CHCl_3$, c = 0.489). IR: 3320s, 2980m, 2940m, 1725s, 1660s, 1540s, 1500m, 1460s, 1420m, 1340s, 1200m, 1130s, 700s, 640m. ^{1}H-NMR: 7.35–7.3, 7.3–7.20, 7.15–7.1 (3m, 10 arom. H), 6.84 (s, NH), 5.44 (br s, NH), 5.02 (s, PhCH_2O), 3.36, 3.29 (AB, *J* = 13.7, PhCH_2), 1.57, 1.45 (2s, 3 Me). ^{13}C-NMR: 176.9, 174.1 (2s, 2 CO), 155.4 (s, OCONH), 136.0, 135.8 (2s, 2 arom. C), 130.2, 128.5, 128.2, 128.0, 127.0 (5d, 10 arom. CH), 67.0 (t, Ph*C*H_2O), 60.6, 57.1 (2s, N*C*MeCH_2Ph and N*C*Me_2), 41.5 (t, Ph*C*H_2), 25.5, 25.2, 22.9 (3q, 3 Me). ESI-MS: 399 ($[M+1]^+$).

*Z-Aib-(*S*)-(αMe)Phe-Aib-N(Me)Ph* ((*S*)-**10**). According to *GP 1*, a mixture of 405 mg (1.02 mmol) (*S*)-**8** and 182 mg (1.05 mmol) azirine **9** in 5 mL of CH_2Cl_2 was stirred for 1 h at 0 °C. Chromatographic workup (CC, SiO_2, CH_2Cl_2/MeOH 19:1) gave 537 mg (92%) of (*S*)-**10** as foam, which was purified by TLC (SiO_2, CH_2Cl_2/MeOH 19:1). IR: 3320s (br), 3060m, 3020m, 2980m, 2930m, 1705s, 1660s, 1640s, 1590m, 1525s, 1495s, 1450s, 1390m, 1360s, 1265s, 1195m, 1170m, 1090s, 1070s, 700s. ^{1}N-NMR: 7.52 (s, NH), 7.4–7.25, 7.2–7.1 (2m, 15 arom. H), 6.43 (br s, NH), 5.43 (br s, NH), 5.06, 4.89 (AB, *J* = 12.3, PhCH_2O), 3.44, 3.13 (AB, *J* = 13.9, PhCH_2), 3.35 (s NMe), 1.47, 1.46, 1.45, 1.44, 1.38 (5s, 5 Me). ^{13}C-NMR: 173.6, 172.9, 172.2 (3s, 3 amide CO), 155.4 (s, OCONH), 145.3, 136.3, 136.1 (3s, 3 arom. C), 130.6, 129.2, 128.6, 128.4, 128.2, 128.0, 127.8, 127.2, 126.9 (9d, 15 arom. CH), 66.9 (t, Ph*C*H_2O), 60.2, 58.0, 57.4 (3s, 3 C(α)), 41.8 (t, Ph*C*H_2), 40.7 (q, NMe), 25.8, 25.6, 25.4, 25.3, 24.1 (5s, 5 Me). CI-MS: 466 (100, $[M–C_7H_7O+1]^+$), 358 (46), 108 (30).

Z-Aib-(S)*-(αMe)Phe-Aib-OH* ((*S*)-**11**). According to *GP 2*, a solution of 353 mg (0.62 mmol) (*S*)-**10** in 2 mL of THF was treated with 2 mL of 6N HCl at 40 °C for 2 h. After extraction and evaporation of the solvent, 270 mg (91%) of (*S*)-**11** was obtained as foam. IR: 3320s (br), 3060m, 3020m, 2980m, 2930m, 17o5s, 1660s, 1530s (br), 1455s, 1380s, 1365m, 1320m, 1265s, 1175m, 1090m, 1075m, 745m, 700s. ^{1}H-NMR: 7.39 (s, NH), 7.35–7.3, 7.3–7.2, 7.1–7.05 (3m, 10 arom. H), 6.68 (br s, NH), 5.77 (br s, NH),5.03, 4.88 (AB, *J* = 12.2, PhC*H*$_2$O), 3.26, 2.99 (AB, *J* = 14.2, PhC*H*$_2$), 1.53, 1.49, 1.46, 1.41, 1.37 (5s, 5 Me). ^{13}C-NMR: 175.7, 174.5, 173.7 (3s, 3 CO), 155.8 (s, OCONH), 135.9, 135.6 (2s, 2 arom. C), 130.6, 128.6, 128.5, 128.3, 128.1, 127.1 (6d, 10 arom. CH), 67.9 (s, C(α)), 67.3 (t, Ph*C*H$_2$O), 59.7, 57.5 (2s, 2 C(α)), 42.3 (t, Ph*C*H$_2$), 25.6, 25.3, 25.1, 24.8, 23.4 (5q, 5 Me). ESI-MS: 506 ([M+Na]$^+$), 484 ([M+1]$^+$).

Z-Aib-(S)*-(αMe)Phe-Aib-*(S)*-(αMe)Phe-N(Me)PhEt* ((*S*,*S*,*R*)-**12**). According to *GP 1*, a mixture of 90 mg (0.186 mmol) (*S*)-**11** and 60 mg (0.216 mmol) azirine (*S*,*R*)-**2a** in 2.5 mL of $CHCl_3$ was stirred at r.t. for 4 d. CC (SiO_2, CH_2Cl_2/MeOH 95:1) yielded 125 mg (88%) of (*S*,*S*,*R*)-**12**). M.p. 233–235 °C (AcOEt/hexane). [α]$_D$ –24.2 ($CHCl_3$, c = 0.124). IR: 3390m, 3250s, 3200s, 3060m, 3025m, 2980m, 2930m, 1705s, 1680s, 1660s, 1530s (br), 1450m, 1440m, 1270s, 1235m, 1185m, 1085m, 745m, 700s. ^{1}H-NMR: 7.35–7.1, 6.95–6.9 (2m, 20 arom. H and 1 NH), 6.17 (s, NH), 6.08 (q, *J* = 6.8, NC*H*MePh), 4.97 (s, NH), 4.93, 4.77 (AB, *J* = 12.2, PhC*H*$_2$O), 3.44, 3.36 (AB, *J* =13.8 PhC*H*$_2$), 3.18, 2.85 (AB, *J* = 14, PhC*H*$_2$), 2.73 (s, NMe), 1.41 (d, *J* = 7, NCH*Me*Ph), 1.56, 1.47, 1.38, 1.23, 1.15, 1.07 (6s. 6 Me).[4]) ESI-MS: 785 ([M+Na]$^+$). Suitable crystals for the X-ray crystal structure determination were obtained from AcOEt/hexane.

[4]) The solubility of the (*S*,*S*,*R*)-**12** was very low so that no reasonable ^{13}C-NMR spectrum was obtained.

Z-Aib-(S)*-(αMe)Phe-Aib-*(R)*-(αMe)Phe-N(Me)PhEt* ((*S*,*R*,*R*)-**12**). According to *GP 1*, a mixture of 90 mg (0.186 mmol) (*S*)-**11** and 60 mg (0.216 mmol) azirine (*R*,*R*)-**2a** in 2.5 mL of $CHCl_3$ was stirred at r.t. for 4 d. CC (SiO_2, CH_2Cl_2/MeOH 95:1) yielded 131 mg (92%) of (*S*,*R*,*R*)-**12**). M.p. 170–172 °C (AcOEt/petroleum ether). [α]$_D$ –6.8 ($CHCl_3$, c = 0.133). IR: 3320s, 3220m, 3060m, 3025m, 2980m, 2935m, 1725s, 1685s, 1660s, 1620s, 1540s, 1515s, 1455s, 1400m, 1380m, 1265s, 1240m, 1185m, 1080s, 745m, 700s. ^{1}H-NMR: 7.3–7.0, 7.0–6.9 (2m, 20 arom. H), 6.82, 6.41 (2s, 2 NH), 6.05 (q, *J* = 6.8, NC*H*MePh), 5.31 (s, NH), 4.88, 4.68 (AB, *J* = 12.2, PhC*H*$_2$O), 3.36 (br s, PhC*H*$_2$), 3.04, 2.76 (AB, *J* = 14, PhC*H*$_2$), 2.70 (s, NMe), 1.41 (d, *J* = 7, NCH*Me*Ph), 1.57, 1.47, 1.31, 1.29, 1.23, 1.00 (6s, 6 Me). ^{13}C-NMR: 173.8, 173.4, 171.9, 171.7 (4s, 4 CO), 155.4 (s, OCONH), 141.3, 137.2, 135.7, 135.3 (4s, 4 arom. C), 131.1, 130.3, 128.6, 128.5, 128.5, 128.3, 128.0, 127.6, 127.1, 126.6, 126.2 (11d, 20 arom. CH), 67.2 (t, Ph*C*H$_2$O), 60.2, 59,5, 57.7, 57.3 (4s, 4 C(α)), 51.9 (d, N*C*HMePh), 43.6, 42.6 (2t, 2 Ph*C*H$_2$), 30.5 (q, NMe), 26.9, 25.9, 24.8, 24.4, 23.2, 23.0, 14.8 (7q, 7Me). ESI-MS: 785 ([M+Na]$^+$). Suitable crystals for the X-ray crystal structure determination were obtained from AcOEt/hexane.

4-BrC$_6$H$_4$CO-Aib-(S)*-(αMe)Phe-Aib-*(R)*-(αMe)Phe-N(Me)PhEt* ((*S*,*R*,*R*)-**13**). To a solution of (*S*,*R*,*R*)-**12** (150 mg, 0.197 mmol) in 5 mL of MeOH were added 70 mg Pd/C (10%) and 60 mg ammonium formate (HCO_2NH_4) and the mixture was heated to reflux for 1 h. The suspension was filtered through Celite, the solvent of the filtrate was evaporated, the residue was dissolved in CH_2Cl_2 and extracted with 1N NaOH. The solvent of the organic phase was evaporated, the residue dissolved in AcOEt (2 mL), and Et_3N (0.2 mL) and 50 mg 4-bromobenzoyl chloride were added. The mixture was stirred at r.t. for 1 h and then washed with 1N HCl. Crystallization was achieved by addition of petroleum ether, yielding 144 mg (90%) of (*S*,*R*,*R*)-**13**, which was purified by TLC (SiO_2, CH_2Cl_2/MeOH 95:1). M.p. 200–202 °C (AcOEt/petroleum ether). [α]$_D$ +22 ($CHCl_3$, c = 0.1). IR: 3405m, 3320s (br), 3220m, 3060w, 3025w, 2990m, 2940m, 1735s, 1670s (br), 1625s, 1590s, 1530s (br), 1494s, 1485s, 1455s, 1395m, 1380s, 1370s, 1305m, 1280m, 1240s, 1220m, 1185m, 1115m, 1085s, 1070m, 1050m, 1030m, 1010m, 780m, 760m, 745m, 735m, 700s. ^{1}H-NMR: 7.57, 7.49 (2d, *J* = 13.6, 4 arom. H), 7.42 (s, NH), 7.25–6.85 (m, 15 arom. H), 6.31 (s, NH), 5.96 (q, *J* = 7, NC*H*MePh), 3.36, 3.30 (AB, *J* = 14, PhC*H*$_2$), 3.04, 2.72 (AB, *J* = 14, PhC*H*$_2$), 2.71 (s, NMe), 1.60, 1.48 (2s, 2 Me), 1.4–1.35 (m, NCH*Me*Ph and 1 Me), 1.34, 1.29, 1.12 (3s, 3 Me). ESI-MS: 834, 832 ([M+Na]$^+$). Suitable crystals for the X-ray crystal structure determination were obtained from AcOEt/petroleum ether.

Synthesis of tetrapeptide amides (*S*,*S*)-**16**, (*S*,*R*)-**16**, and (*S*,*R*)-**17** *with accumulated (αMe)Phe units*

Z-Aib-(S)*-(αMe)Phe-*(S)*-(αMe)Phe-NMe$_2$* ((*S*,*S*)-**15**) *and Z-Aib-*(S)*-(αMe)Phe-*(R)*-(αMe)Phe-NMe$_2$* ((*S*,*R*)-**15**). According to *GP 1*, a mixture of 399 mg (1.00 mmol) (*S*)-**8** in10mL of CH_2Cl_2 and 200 mg (1.06 mmol) of azirine rac-**14** in 5 mL of CH_2Cl_2 was stirred at 0 °C for 2 h. Evaporation of the solvent and purification by CC (AcOEt) yielded 560 mg (96%) of a mixture of (*S*,*S*)- and (*S*,*R*)-**15**. Separation of the diastereomers by MPLC (AcOEt) gave 215 mg (37%) (*S*,*S*)-**15** and 205 mg (35%) (*S*,*R*)-**15**, which were crystallized from Et_2O/hexane/CH_2Cl_2. (*S*,*R*)-**15**: M.p. 102–102.5 °C. IR: 3300m, 3060w, 2980w, 1705s, 1660s, 1630s, 1530s, 1495s, 1450m, 1265s, 1090m,

735m, 700s. ^{1}H-NMR: 7.68 (s, NH), 7.5–7.25 (m, 15 arom. H), 6.49, 5.55 (2s, 2 NH), 5.24, 5.08 (AB, *J* = 12.4, PhC*H*$_2$O), 3.75–3.65 (m, PhC*H*$_2$), 3.33, 3.25 (AB, *J* = 14, PhC*H*$_2$), 3.22 (br s, NMe$_2$), 1.65, 1.61, 1.56, 1.53 (4s, 4 Me). ^{13}C-NMR: 173.2, 172.2, 171.3 (3s, 3 CO), 155.3 (s, OCONH), 136.6, 136.3, 136.0 (3s, 3 arom. C), 130.7, 130.5, 128.6, 128.3, 128.2, 127.9, 127.8, 126.9, 126.5 (9d, 15 arom. CH), 66.9 (t, PhCH$_2$O), 65.7, 60.4, 57.3 (3s, 3 C(α)), 42.6, 41.1 (2t, 2 PhCH$_2$), 38.0 (q, NMe$_2$), 25.8, 24.7, 24.0, 23.1 (4q, 4 Me). CI-MS: 587 (5, [M+1]$^+$), 542 (100, [M–NMe$_2$+1]$^+$).
(*S*,*S*)-**15**: M.p. 94–98 °C. ^{1}H-NMR: 7.69 (s, NH), 7.45–7.15 (m, 15 arom. H), 6.72, 5.38 (2s, 2 NH), 5.02, 5.0.978 (AB, *J* = 14, PhC*H*$_2$O), 3.59, 3.31 (AB, *J* = 14.0, PhC*H*$_2$), 3.46, 3.28 (AB, *J* = 13.9, PhC*H*$_2$), 3.16 (br s, NMe$_2$), 1.69, 1.57, 1.54, 1.50 (4s, 4 Me). ^{13}C-NMR: 172.9, 171.8, 171.4 (3s, 3 CO), 155.2 (s, OCONH), 136.6, 136.1, 135.8 (3s, 3 arom. C), 130.5, 130.4, 128.5, 128.2, 128.1, 127.9, 126.8, 126.6 (8d, 15 arom. CH), 66.9 (t, Ph*C*H$_2$O), 60.7, 60.5, 57.3 (3s, 3 C(α)), 42.1, 41.9 (2t, 2 Ph*C*H$_2$), 38.2 (q, NMe$_2$), 25.3, 24.2, 23.2 (3q, 4 Me).

*Z-Aib-(*S*)-(αMe)Phe-(*R*)-(αMe)Phe-Aib-N(Me)Ph* ((*S*,*R*)-**16**). According to *GP 2*, to a solution of 140 mg (0.239 mmol) of (*S*,*R*)-**15** in 1 mL THF was added 1 mL of 6N HCl and the mixture was stirred at 40 °C for 2.5 h. The solution was extracted with CH_2Cl_2 and water and the organic solvent was evaporated. The formation of the peptide acid was confirmed by the spectroscopic data. ^{1}H-NMR: 7.3–7.2, 6.95–6.9 (2m, 15 arom. H), 6.42, 5.12 (2s, 2 NH), 4.95, 4.92 (AB, *J* = 12, PhC*H*$_2$O), 3.50, 3.34 (AB, *J* = 13.7, PhC*H*$_2$), 3.16, 3.07 (AB, *J* = 13.7, PhC*H*$_2$), 1.47, 1.39, 1.37, 1.33 (4s, 4 Me). ^{13}C-NMR: 174.8, 174.2, 173.9 (3s, 3 CO), 155,5 (s, OCONH), 136.3, 135.6, 135.3 (3s, 3 arom. C), 130.9, 130.6, 128.8, 128.6, 128.5, 128.1, 127.3, 126.8 (8d, 15 arom. CH), 67.4 (t, Ph*C*H$_2$O), 60.9, 59.5, 57.4 (3s, 3 C(α)), 42.2, 41.2 (2t, 2 Ph*C*H$_2$), 25.5, 25.0, 23.0, 22.6 (4q, 4 Me). ESI-MS: 582 ([M+Na]$^+$), 560 ([M+1]$^+$).
According to *GP 1*, the peptide acid was dissolved in 2 mL of $CHCl_3$ and treated with 50 mg (0.287 mmol) of azirine **9** at r.t. for 2 d. Chromatographic workup (CC, CH_2Cl_2/MeOH 19:1) gave 161 mg (92%) of (*S*,*R*)-**16** as a colorless solid. M.p. 96–97 °C. [α]$_D$ +25.6 ($CHCl_3$, c = 0.11). IR: 3300s (br), 3060w, 3025m, 2980m, 2930m, 1710s, 1680s, 1660s, 1595m, 1530s (br), 1495s, 1455s, 1390m, 1365m, 1265s, 1090s, 740m, 705s. ^{1}H-NMR: 7.61 (s, NH), 7.25–7.1 (m, 19 arom. H), 6.78 (s, NH), 6.7–6.65 (m, 1 arom. H), 6.40, 5.08 (2s, 2 NH), 4.98, 4.74 (AB, *J* = 12, PhC*H*$_2$O), 3.74, 3.21 (AB, *J* = 13.8, PhC*H*$_2$), 3.37 (s, NMe), 2.92, 2.62 (AB, *J* = 13.7, PhC*H*$_2$), 1.51, 1.49, 1.43, 1.34, 1.31, 1.10 (6s, 6 Me). ^{13}C-NMR: 173.6, 173.5, 173.4, 172.6 (4s, 4 CO), 155.3 (s, OCONH), 146.0, 137.5, 135.6, 134.9 (4s, 4 arom. C), 131.2, 130.2, 128.9, 128.6, 128.5, 127.7, 127.3, 126.4 (8d, 20 arom. CH), 67.2 (t, Ph*C*H$_2$O), 60.6, 59.0, 57.5, 57.1 (4s, 4 C(α)), 43.9, 39.9 (2t, 2 Ph*C*H$_2$), 40.1 (q, NMe$_2$), 26.2, 25.8, 24.2, 23.9, 21.0 (5q, 6 Me). ESI-MS: 757 ([M+Na]$^+$).

*Z-Aib-(*S*)-(αMe)Phe-(*S*)-(αMe)Phe-Aib-N(Me)Ph* ((*S*,*S*)-**16**). According to *GP 2*, 124 mg (0.211 mmol) of (*S*,*S*)-**15** in 2 mL THF were hydrolysed with 1 mL of 6N HCl at 40 °C for 4 h. The solution was extracted with CH_2Cl_2 and water and the organic solvent was evaporated. According to *GP 1*, the residue was dissolved in $CHCl_3$ (2 mL) and reacted with 50 mg (0.287 mmol) of azirine **9** at r.t. for 2 d. Crystallisation from AcOEt/petroleum ether gave 139 mg (90%) of (*S*,*S*)-**16**. M.p. 187–189 °C. [α]$_D$ –131.3 ($CHCl_3$, c = 0.094). IR: 3350s, 3290s, 3065w, 3025w, 2995m, 2940m, 1715s, 1670s, 1645s, 1595w, 1530s, 1495s, 1450m, 1390m, 1365m, 1265s, 1085s, 770m, 750m, 705s. ^{1}H-NMR: 7.57 (s, NH), 7.35–7.3, 7.25–7.15, 7.1–7.0 (3m, 20 arom. H and 1 NH), 6.00, 5.21 (2s, 2 NH), 4.92, 4.70 (AB, *J* = 12.0, PhC*H*$_2$O), 3.85, 3.50 (AB, *J* = 13.8, PhC*H*$_2$), 3.45 (s, NMe), 3.23, 3.19 (AB, *J* = 14.1, PhC*H*$_2$), 1.60, 1.59, 1.47, 1.44, 1.32, 1.13 (6s, 6 Me). ^{13}C-NMR: 173.9, 173.8, 173.7, 172.4 (4s, 4 CO), 155.4 (s, OCONH), 146.0, 137.7, 136.6, 135.5 (4s, 4 arom. C), 131.3, 131.0, 128.9, 128.7, 128.5, 128.3, 128.0, 127.6, 127.5, 126.8, 126.5, 126.3 (12d, 20 arom. CH), 67.2 (t, Ph*C*H$_2$O), 60.4, 59.7, 57.6, 57.4 (4s, 4 C(α)), 40.1 (q, NMe$_2$), 39.2, 39.1 (2t, 2 Ph*C*H$_2$), 27.0, 26.6, 25.9, 24.6, 23.7 (5q, 6 Me). ESI-MS: 757 ([M+Na]$^+$). Suitable crystals for the X-ray crystal structure determination were obtained from AcOEt/hexane.

*4-BrC$_6$H$_4$CO-Aib-(*S*)-(αMe)Phe-(*R*)-(αMe)Phe-Aib-N(Me)Ph* ((*S*,*R*)-**17**). A mixture of 73 mg (0.10 mmol) of (*S*,*R*)-**16**, 70 mg Pd/C (10%), and 60 mg ammonium formate (HCO_2NH_4) in 7 mL MeOH was heated to reflux for 15 min. The suspension was filtered (Celite), the solvent evaporated and the residue dissolved in AcOEt. Then, 0.6 mL Et_3N and 65 mg 4-bromobenzoyl chloride were added and the mixture was stirred at 30 °C for 2 h. Extraction with 1N HCl and 1N NaOH, drying of the organic phase ($MgSO_4$), evaporation of the solvent, and purification by CC (CH_2Cl_2/MeOH 19:1) yielded 50 mg (64%) of (*S*,*R*)-**17**. M.p. 218–222 °C. [α]$_D$ –18.7 ($CHCl_3$, c = 0.118). IR: 3350s, 3290s, 3065w, 3025w, 2995m, 2940m, 1715s, 1670s, 1645s, 1595w, 1530s, 1495s, 1450m, 1390m, 1365m, 1265s, 1085s, 770m, 750m, 705s. ^{1}H-NMR: 7.65 (s, NH), 7.51, 7.45 (AB, *J* = 8.5, 4 arom. H), 7.25–7.0 (m, 13 arom. H and 2 NH), 6.85–6.8 (m, 2 arom. H), 6.32 (s, NH), 3.70, 3.20 (AB, *J* = 13.9, PhC*H*$_2$), 3.32 (s, NMe), 2.93, 2.81 (AB, *J* = 13.8, PhC*H*$_2$), 1.47, 1.46, 1.44, 1.37, 1.35, 1.27 (6s, 6 Me). ^{13}C-NMR: 174.0, 173.9, 173.8, 172.7 (4s, 4 CO), 165.4 (s, ArCONH), 145.9, 137.4, 135.2, 131.8, 127.2 (5s, 5 arom. C), 131.9, 131.6, 131.1, 130.3, 129.2, 129.0, 128.6, 128.0, 127.8, 127.5, 127.4, 126.6, 126.5 (13d, 19 arom. CH), 60.6, 59.1, 57.6, 57.4 (4s, 4 C(α)), 43.5, 40.5 (2t, 2 Ph*C*H$_2$), 40.2 (q, NMe$_2$), 26.0, 25.9, 25.7, 24.1, 24.0, 22.0 (6q, 6 Me). ESI-MS: 806, 804 ([M+Na]$^+$). Suitable crystals for the X-ray crystal structure determination were obtained from AcOEt/CH_2Cl_2.

Synthesis of tetrapeptide amides (*R*,*S*,*R*)-**20** and (*R*,*R*,*R*)-**20** *with accumulated Aib units*

*(*R,S*)-4-Bromo-*N*-{1-benzyl-1-methyl-2-[2-(1-methoxy-1-methylethyl)pyrrolidin-1-yl]benzamide* ((*R*,*S*)-**18**. To a solution of 201 mg (1 mmol) 4-bromobenzoic acid in 2 mL of THF at 0 °C was slowly added a solution of 286 mg (1 mmol) of the azirine (*R*,*S*)-**3** in 2 mL of THF. The mixture was stirred at r.t. overnight and then heated to 67 °C for 3 h. Then, the solvent was evaporated, the residue dissolved in CH_2Cl_2 and washed with 5 mL of 1N NaOH. The solvent was evaporated and the residue crystallized from AcOEt/hexane to give 221 mg (45%) of (*R*,*S*)-**18**. $[\alpha]_D$ –20.7 ($CHCl_3$, c = 1.011). IR: 3290s, 2980s, 2940m, 1665s, 1605s, 1540s, 1475m, 1450m, 1400s, 1300s, 1245m, 1145s, 1060m, 1015m, 850m, 705s. ^{1}H-NMR: 7.55–7.50 (m, 4 arom. H), 7.4 (br, NH), 7.2–7.15, 7.0–6.95 (2m, 5 arom. H), 4.53 (d, *J* = 8, CHN), 4.1–4.05 (m, 1 H), 3.78, 3.40 (AB, *J* = 14, PhC*H*$_2$), 3.5–3.35 (m, 1 H), 3.17 (s, MeO), 2.1–2.0 (m, 2 H), 1.9–1.8 (m, 5 H), 1.20, 1.14 (2s, 2 Me). ^{13}C-NMR: 171.3, 164.8 (2s, 2 CO), 136.7, 134.2 (2s, 2 arom. C), 131.6, 129.9, 128.3, 128.0, 125.8 (5d, 9 arom. CH), 126.7 (s, 1 arom. C), 78.5 (s, Me_2*C*), 65.2 (d, CHN), 61.6 (s, C(α)), 49.1 (q, MeO), 48.0, 39.8 (2t, Ph*C*H_2, CH_2N), 25.2, 23.6 (2t, 2 CH_2), 22.6, 22.3, 21.7 (3q, 3 Me). CI-MS: 489, 487 ($[M+1]^+$). Suitable crystals for the X-ray crystal structure determination were obtained from AcOEt/hexane.

*4-BrC$_6$H$_4$CO-(*R*)-(αMe)Phe-Aib-Aib-(*S*)-(αMe)Phe-N(Me)PhEt* ((*R*,*S*,*R*)-**20**) *and 4BrC$_6$H$_4$CO-(*R*)-(αMe)Phe-Aib-Aib-(*R*)-(αMe)Phe-N(Me)PhEt* ((*R*,*R*,*R*)-**20**). According to *GP 2*, 125 mg (0.25 mmol) (*R*,*S*)-**18** were dissolved in 1 mL THF, 1 mL of 6N HCl was added and the mixture stirred at 40 °C overnight. The resulting solution was extracted with CH_2Cl_2 and water, dried, and the solvent evaporated. The residue was dissolved in 5 mL of CH_2Cl_2, 50 mg (0.28 mmol) azirine **9** were added according to *GP 1* and the mixture was stirred at r.t. for 2 h. Then, the solvent was evaporated, the residue dissolved in AcOEt and the product precipitated by addition of petroleum ether. The solid material was again dissolved in 1 mL of THF and hydrolysed with 6N HCl at 40 °C for 30 min according to *GP 2*. After usual workup, the residue was dissolved in 4 mL of CH_2Cl_2 and according to *GP 1* reacted with 55 mg (0.31 mmol) azirine **9** at r.t. for 1 h. The solution was concentrated, the residue was dissolved in AcOEt and the product precipitated by addition of petroleum ether to give 120 mg (77%) of *4-BrC$_6$H$_4$CO-(*R*)-(αMe)Phe-Aib-Aib-N(Me)Ph* ((*R*)-**19**). ^{1}H-NMR: 7.5–7.45, 7.35–7.25, 7.2–7.15, 7.05–7.0 (4m, 14 arom. H and 1 NH), 6.89, 6.73 (2s, 2 NH), 4.53 (d, *J* = 8, CHN), 3.40, 3.23 (AB, *J* = 13.8, PhC*H*$_2$), 3.24 (s, NMe), 1.59, 1.44, 1.42, 1.40 (4s, 5 Me). ESI-MS: 645, 643 ($[M+Na]^+$).
According to *GP 2*, a solution of 100 mg (0.161 mmol) tripeptide amide (*R*)-**19** in 2 mL of THF was treated with 2 mL of 6N HCl at 40 °C for 1 h. After usual workup, the tripeptide acid was dissolved in 5 mL of CH_2Cl_2, and 2.5 mL of the solution was reacted with 30 mg (0.108 mmol) azirine (*S*,*R*)-**2a** and (*R*,*R*)-**2a**, respectively, at r.t. overnight. Because the reactions were incomplete, the solvent was evaporated, each residue was dissolved in 1.5 mL of $CHCl_3$ and the solutions stirred at r.t. for 3 d. Then, the solvent was again removed and the residue crystallized in each case.

*4-BrC$_6$H$_4$CO-(*R*)-(αMe)Phe-Aib-Aib-(*S*)-(αMe)Phe-N(Me)PhEt* ((*R*,*S*,*R*)-**20**). Yield: 62 mg (95%). M.p. 174–176 °C ($CHCl_3$/AcOEt/petroleum ether). $[\alpha]_D$ +59.0 ($CHCl_3$, c = 0.105). IR: 3300s (br), 3065w, 3025w, 2995m, 2940m, 1670s (br), 1640s, 1620s, 1540s, 1500s, 1480s, 1455s, 1395m, 1380s, 1360m, 1320m, 1230m, 1180m, 1070m, 755m, 700s. ^{1}H-NMR: 7.6–7.55, 7.3–7.25, 7.15–7.05 (3m, 19 arom. H and 3 NH), 6.53 (s, NH), 6.0–5.95 (m, NC*H*MePh), 3.41, 3.03 (AB, *J* = 14, PhC*H*$_2$), 3.04, 2.76 (AB, *J* = 14, PhC*H*$_2$), 3.32, 3.27 (AB, *J* = 14.3, PhC*H*$_2$), 2.83 (s, NMe), 1.64, 1.54, 1.39, 1.36 (4s, 4 Me), 1.34 (br, NCH*Me*Ph), 1.32, 1.28 (2s, 2 Me). ^{13}C-NMR: 173.9, 173.4, 173.1, 171.8, 167.0 (5s, 5 CO), 140.8, 137.4, 136.8, 132.0, 128.1 (5s, 5 arom. C), 132.1, 131.1, 130.7, 128.9, 128.4, 128.0, 127.8, 127.3, 127.0, 126.3 (10d, 19 arom. CH), 60.4, 60.2, 57.5, 57.0 (4s, 4 C(α)), 52.2 (d, N*C*HMePh), 42.5, 40.7 (2t, 2 Ph*C*H_2), 30.3 (q, NMe), 26.7, 26.0, 25.2, 24.6, 23.2, 22.8, 14.9 (6q, 7Me). ESI-MS: 834, 832 ($[M+Na]^+$).

*4-BrC$_6$H$_4$CO-(*R*)-(αMe)Phe-Aib-Aib-(*R*)-(αMe)Phe-N(Me)PhEt* ((*R*,*R*,*R*)-**20**). Yield: 56 mg (87%). M.p. 132–134 °C (MeOH/AcOEt/petroleum ether). ^{1}H-NMR (MeOD): 7.85–7.8, 7.75–7.7, 7.65–7.6, 7.35–7.3, 7.3–7.2, 7.2–7.15, 7.1–6.95 (7m, 19 arom. H), 5.94 (q, *J* = 7, NC*H*MePh), 3.38 (s, PhC*H*$_2$), 3.20, 2.85 (AB, *J* = 13.5, PhC*H*$_2$), 2.80 (s, NMe), 1.62, 1.59, 1.57 (3s, 3 Me), 1.48 (d, *J* = 7, NCH*Me*Ph), 1.43, 1.36, 1.31 (3s, 3 Me). ^{13}C-NMR (MeOD): 182.1, 176.3, 176.1, 173.6, 168.6 (5s, 5 CO), 141.6, 137.7, 137.3, 133.9, 128.9 (5s, 5 arom. C), 132.7, 132.1, 132.0, 130.4, 129.2, 128.9, 128.5, 128.1, 127.7, 127.6, 127.5 (11d, 19 arom. CH), 61.9, 60.8, 58.4, 57.9 (4s, 4 C(α)), 53.6 (d, N*C*HMePh), 43.8, 40.2 (2t, 2 Ph*C*H_2), 31.4 (q, NMe), 28.3, 27.1, 24.6, 23.8, 23.6, 23.2, 15.1 (7q, 7Me).

X-ray Crystal-Structure Determination of (*R*,*S*)-**18**, (*S*,*S*,*R*)-**12**, (*S*,*R*,*R*)-**12**, (*S*,*R*,*R*)-**13**, (*S*,*S*)-**16** and (*S*,*R*)-**17** (see *Table 3* and *Figs. 2 – 7*)[5]).

The measurements were made at low temperature using graphite-monochromated Mo$K\alpha$ radiation (λ 0.71073 Å) on a *Rigaku AFC5R* diffractometer fitted to a 12-kW rotating-anode generator. The intensities of three standard reflections were measured after every 150 reflections and remained stable throughout the data collection. The intensities were corrected for *Lorentz* and polarization effects. Azimuthal scans of several reflections indicated no need for an absorption correction. Equivalent reflections, other than *Friedel* pairs, were merged. The data collection and refinement parameters are given in *Table 3* and views of the molecules are shown in Figs. 2 – 7. The structures were solved as described for the individual structures below. For all structures, the non-H-atoms were refined anisotropically. Unless indicated otherwise below, amide H-atoms were generally placed in the positions indicated by difference electron density maps and their positions were allowed to refine together with individual isotropic displacement parameters. Otherwise, and for all other H-atoms, they were placed in geometrically calculated positions and refined by using a riding model, with $U_{iso}(H) = 1.2U_{eq}$(parent atom) ($1.5U_{eq}$ for Me groups). The refinement of each structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function $\Sigma w(F_o^2 - F_c^2)^2$. The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. Neutral atom scattering factors for non-hydrogen atoms were taken from Maslen, Fox and O'Keefe[75], and the scattering factors for H-atoms were taken from Stewart, Davidson and Simpson[76]. Anomalous dispersion effects were included in F_c[77]; the values for f' and f'' were those of Creagh and McAuley[78]. The values of the mass attenuation coefficients are those of Creagh and Hubbel[79]. The *SHELXL-2018* program[80] was used for all calculations.

The structure of (*R*,*S*)-**18** was solved by Patterson methods using *SHELXS86*[81], which revealed the position of the Br-atom. All remaining non-hydrogen atoms were located in a Fourier expansion of the Patterson solution. Refinement of the absolute structure parameter[82, 83] yielded a value of -0.026(7), which confidently confirms that the refined model represents the true enantiomorph.

The structure of (*S*,*S*,*R*)-**12** was solved by direct methods using *SHELXS86*, which revealed the positions of all non-hydrogen atoms. One reflection, whose intensity was considered to be an extreme outlier, was omitted from the final refinement. The absolute configuration could not be determined reliably and, therefore, the enantiomer used in the refinement was based on the known (5*S*,14*R*)-configuration of the molecule.

The structure of (*S*,*R*,*R*)-**12** was solved by using the fragment orientation and translation routines of *DIRDIF92*[84], which revealed the positions of most non-hydrogen atoms. The starting fragment consisted of three peptide units from the backbone of the related structure of (*S*,*S*,*R*)-**12**. All remaining non-hydrogen atoms were located in a subsequent difference electron density map. The crystal was quite weakly diffracting and of sub-standard quality with some of the reflection profiles exhibiting shoulders. The number of observed reflections and the precision of the geometric parameters are quite low, but the structure is clearly defined. The enlarged atomic displacement parameters for some atoms may in part be due to the influence of the poor crystal on the data. The asymmetric unit contains two molecules of the peptide plus two ethyl acetate molecules. The atomic coordinates of the molecules were tested carefully for a relationship from a higher symmetry space group using the program *PLATON*[85], but none could be found. Peptide molecule B exhibits disorder of the terminal methylbenzyl group and of the terminal benzoyl ring. The ethyl groups of the solvent molecules are also disordered. Two sets of positions were defined for the ring atoms of both disordered groups in molecule B, plus the methyl group of the methylbenzyl group, and the ring atoms were constrained to an ideal hexagon. The site occupation factors of the major conformations of these groups refined to 0.611(9) and 0.555(17), respectively. Similarity restraints were applied to the chemically equivalent bond lengths involving all disordered C-atoms that were not constrained, while neighboring atoms within and between each conformation of the disordered butyl groups were restrained to have similar atomic displacement parameters. The amide H-atoms were included as riding in calculated positions. Five reflections, whose intensities were considered to be extreme outliers, were omitted from the final refinement. The absolute configuration could not be determined reliably and, therefore, the enantiomer used in the refinement was based on the known (5*S*)-configuration of the molecule.

The diffracting power of the crystal of (*S*,*R*,*R*)-**13** fell off sharply with increasing diffraction angle and no discernible diffraction intensities were observed for $2\theta > 50°$.The structure was solved by Patterson methods using *SHELXS86*, which revealed the position of the Br-atom. All remaining non-hydrogen atoms were located in a Fourier expansion of the Patterson solution. The asymmetric unit contains one molecule of the peptide plus one disordered AcOEt molecule. The solvent disorder could not be modelled satisfactorily, so an ordered model with enlarged atomic displacement ellipsoids was employed. The amide H-atoms were included as riding in calculated positions. Three

[5]) CCDC-1992397-1992402 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/structures.

reflections, whose intensities were considered to be extreme outliers, were omitted from the final refinement. Refinement of the absolute structure parameter yielded a value of -0.001(8), which confidently confirms that the refined model represents the true enantiomorph.

The structure of (*S*,*S*)-**16** was solved using the fragment orientation and translation routines of *DIRDIF92*, which revealed the positions of most non-hydrogen atoms. The starting fragment consisted of three peptide units from the backbone of the related structure of (*S*,*S*,*R*)-**12**. All remaining non-hydrogen atoms were located in a subsequent difference electron density map. The asymmetric unit contains two molecules of the peptide plus one water molecule with full site occupancy and one water molecule which seems to be present only in approximately 30% of its sites and it site occupancy was fixed at 0.3. The atomic coordinates of the molecules were tested carefully for a relationship from a higher symmetry space group using the program *PLATON*, but none could be found. The positions of the H-atoms of the water molecules could not be determined reliably and were not included in the model. The absolute configuration could not be determined unequivocally and, therefore, the enantiomer used in the refinement was based on the configuration of the molecule known from the synthesis.

The diffracting power of the crystal of (*S*,*R*)-**17** fell off sharply with increasing diffraction angle and no discernible diffraction intensities were observed for $2\theta > 50°$. The structure was solved by Patterson methods using *DIRDIF92*, which revealed the positions of the Br-atoms. All remaining non-hydrogen atoms were located in several steps through Fourier expansions of the partial solution. The asymmetric unit contains two independent molecules of the peptide plus one ordered and one disordered molecule of AcOEt. The atomic coordinates of the molecules were tested carefully for a relationship from a higher symmetry space group using the program *PLATON*, but none could be found. The disordered AcOEt molecule involves end-to-end flipping of the molecule. Two sets of positions were defined for carbonyl O-atom and the methylene C-atom and the site occupation factor of the major conformation of the molecule refined to 0.641(10). Similarity restraints were applied to the chemically equivalent bond lengths involving all disordered atoms, while neighboring atoms within and between each conformation of the molecule were restrained to have similar atomic displacement parameters. The amide H-atoms were included as riding in calculated positions. One reflection, whose intensity was considered to be an extreme outlier, was omitted from the final refinement. Refinement of the absolute structure parameter yielded a value of –0.008(4), which confidently confirms that the refined model represents the true enantiomorph.

Table 3. Crystallographic Data for compounds (*R*,*S*)-**18**, (*S*,*S*,*R*)-**12**, (*S*,*R*,*R*)-**12**, (*S*,*R*,*R*)-**13**, (*S*,*S*)-**16** and (*S*,*R*)-**17**

		(*R*,*S*)-**18**	(*S*,*S*,*R*)-**12**	(*S*,*R*,*R*)-**12**
Crystallized from		AcOEt/hexane	AcOEt/hexane	AcOEt/hexane
Empirical formula		$C_{25}H_{31}BrN_2O_3$	$C_{45}H_{55}N_5O_6$	$C_{45}H_{55}N_5O_6 \cdot C_4H_8O_2$
Formula weight		487.43	761.94	850.04
Crystal color, habit		colorless, needle	colorless, prism	colorless, prism
Crystal dimensions [mm]		0.15 × 0.18 × 0.48	0.18 × 0.20 × 0.50	0.25 × 0.30 × 0.42
Temperature [K]		173(1)	173(1)	173(1)
Crystal system		orthorhombic	orthorhombic	triclinic
Space group		$P2_12_12_1$	$P2_12_12_1$	$P1$
Z		4	4	2
Reflections for cell determination		23	25	22
2θ range for cell determination [°]		35–39	32–40	25–37
Unit cell parameters	a [Å]	11.384(4)	16.770(3)	10.802(8)
	b [Å]	18.452(3)	22.601(8)	12.641(7)
	c [Å]	11.0453(19)	10.918(4)	17.614(8)
	α [°]	90	90	97.17(5)
	β [°]	90	90	92.09(5)
	γ [°]	90	90	100.46(6)
	V [Å^3]	2320.1(10)	4138(2)	2342(2)
$F(000)$		1016	1632	912
D_x [g cm^{-3}]		1.395	1.223	1.205
μ(Mo$K\alpha$) [mm^{-1}]		1.801	0.0820	0.0820
Scan type		ω	ω	ω
$2\theta_{(max)}$ [°]		60.0	50.0	50.2
Total reflections measured		7988	4753	8661
Symmetry independent reflections		6214	4619	8661
R_{int}		0.030	0.037	-
Reflections with $I > 2\sigma(I)$		4401	3750	5913
Reflections used in refinement		6214	4618	8656
Parameters refined; restraints		288; 0	529; 4	1240; 431
Final	$R(F)$ [$I > 2\sigma(I)$ reflections]	0.0417	0.0410	0.0574
	$wR(F^2)$ (all data)	0.0954	0.1146	0.1773
Weighting parameter (a; b)[a]		0.0348; 0	0.0569; 0.4774	0.0787; 2.0554
Goodness of fit		1.023	1.067	1.031
Final Δ_{max}/σ		0.000	0.000	0.000
$\Delta\rho$ (max; min) [e Å^{-3}]		0.41; –0.43	0.23; –0.19	0.43; –0.29

[a] $w^{-1} = \sigma^2(F_o^2) + (aP)^2 + bP$, where $P = (F_o^2 + 2F_c^2)/3$

Table 3. Crystallographic Data for Compounds (*R*,*S*)-**18**, (*S*,*S*,*R*)-**12**, (*S*,*R*,*R*)-**12**, (*S*,*R*,*R*)-**13**, (*S*,*S*)-**16** and (*S*,*R*)-**17** (continued)

		(*S*,*R*,*R*)-**13**	(*S*,*S*)-**16**	(*S*,*R*)-**17**
Crystallized from		AcOEt/petroleum ether	AcOEt/hexane	AcOEt/CH_2Cl_2
Empirical formula		$C_{44}H_{52}BrN_5O_5 \cdot C_4H_8O_2$	$C_{43}H_{51}N_5O_6 \cdot 0.65H_2O$	$C_{42}H_{48}BrN_5O_5 \cdot C_4H_8O_2$
Formula weight		898.92	745.60	870.86
Crystal color, habit		colorless, prism	colorless, prism	colorless, prism
Crystal dimensions [mm]		0.40 × 0.40 × 0.42	0.17 × 0.40 × 0.50	0.20 × 0.38 × 0.45
Temperature [K]		173(1)	173(1)	173(1)
Crystal system		orthorhombic	monoclinic	triclinic
Space group		$P2_12_12_1$	$P2_1$	$P1$
Z		4	4	2
Reflections for cell determination		21	25	25
2θ range for cell determination [°]		33–39	26–36	24–26
Unit cell parameters	a [Å]	19.245(3)	10.913(5)	11.554(3)
	b [Å]	22.139(5)	35.161(8)	11.830(2)
	c [Å]	11.028(4)	11.442(6)	18.188(4)
	α [°]	90	90	87.167(18)
	β [°]	90	111.57(4)	74.47(2)
	γ [°]	90	90	69.928(18)
	V [Å^3]	4698(2)	4083(3)	2247.2(9)
$F(000)$		1896	1594	916
D_x [g cm^{-3}]		1.271	1.213	1.287
μ(Mo$K\alpha$) [mm^{-1}]		0.929	0.0820	0.969
Scan type		ω	ω	ω
$2\theta_{(max)}$ [°]		50.0	55.0	55.1
Total reflections measured		7210	10007	15334
Symmetry independent reflections		6584	9511	14712
R_{int}		0.076	0.025	0.025
Reflections with $I > 2\sigma(I)$		3889	7034	9364
Reflections used in refinement		6581	9511	14711
Parameters refined; restraints		560; 0	1037; 2	1100; 70
Final	$R(F)$ [$I > 2\sigma(I)$ reflections]	0.0539	0.0497	0.0455
	$wR(F^2)$ (all data)	0.1546	0.1406	0.1176
Weighting parameter (a; b)[a]		0.0688; 2.4765	0.0724; 0.3323	0.0388; 1.8090
Goodness of fit		1.019	1.035	1.007
Final Δ_{max}/s		0.000	0.000	0.000
$\Delta\rho$ (max; min) [e Å^{-3}]		0.35; –0.41	0.37; –0.20	0.39; –0.53

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Author Contribution Statement

H. H. and C. B. B. conceived and designed the study, C. B. B. performed the experiments, analyzed the data and depicted the presentation, A. L. carried out the X-ray crystal analysis, and H. H. and A. L. contributed to writing of the manuscript.

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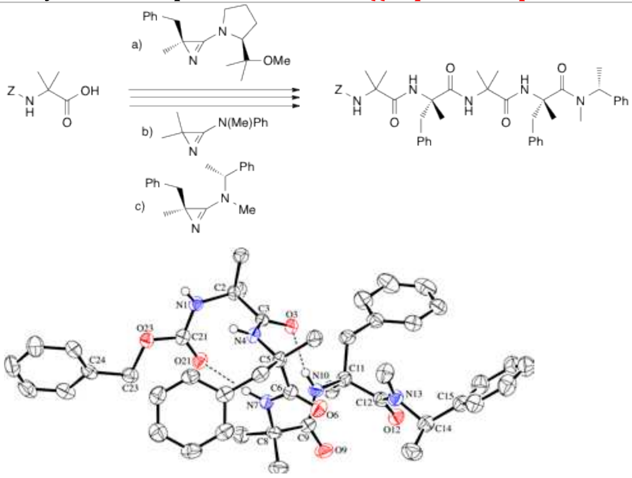

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Sterically congested tetrapeptide amides containing two Aib and two (αMe)Phe unit were prepared via the 'azirine/oxazolone method' and their solid-state conformations determined by X-ray crystallography.